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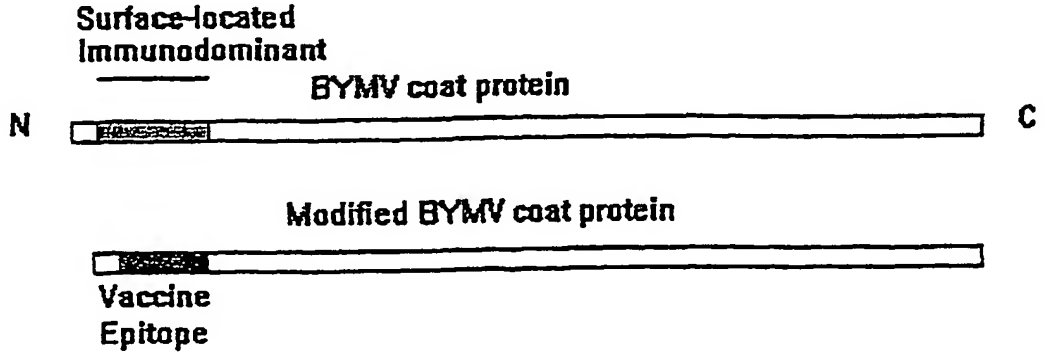

(57) Abstract: Plants infected with potato virus Y (PVY) were inoculated with infectious PVX RNA encoding PVX coat protein (CP) and modified chimeric NDV/BYMV CP. Antigen-coated plate indirect ELISA and immunoelectron microscopy of virus purified from infected plants showed that progeny virions contained from <1% to as much as 25% chimeric CP. The method can be used to produce and purify large amounts of NDV vaccine. Transencapsidated PVY virions expressing NDV were purified and used to induce anti-NDV antibodies in mice. Further, transgenic tobacco plants expressing HIV/BYMV CP were inoculated with BYMV. Progeny virions from transgenic plants contained transgenic HIV/BYMV CP.

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PRODUCTION OF VACCINES USING TRANSGENIC PLANTS OR MODIFIED PLANT VIRUSES AS EXPRESSION VECTORS AND TRANSENCAPSIDATED VIRAL COAT PROTEINS AS EPITOPE PRESENTATION SYSTEMS

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to methods for production of recombinant peptides in plants. More particularly, this invention relates to methods for inserting immunogenic peptides into the coat protein of a plant virus for the purpose of generating quantities of immunogenic peptides for vaccine and antibody production. Further, the invention relates to the recombinant nucleic acid, vectors, and virions used to generate the expressed immunogenic peptides and to the transgenic plant expressing the immunogenic peptides.

Description of the Relevant Art

It is now well established that plants can serve as useful, accessible resources for generating large quantities of foreign gene products efficiently and inexpensively. "Foreign gene" refers to a gene that is not part of the genome of a particular plant. "Foreign gene products" refers to RNAs and proteins that are encoded by the foreign gene. Plants may be used to express foreign gene products or to overexpress endogenous gene products via introduction of a genetically engineered DNA sequence encoding the foreign or endogenous gene into the genetic material of a suitable plant through the use of various biotechnological methods. The term "introduction" refers to a method which is capable of introducing the genetically engineered DNA sequence into the genetic material of a plant cell. Examples of such biotechnological methods are Agrobacterium-mediated transfer, plant virus mediated-transfer, microinjection, microprojectile bombardment, electroporation, PEG-mediated transformation and transformation of plant protoplasts with virus-based stable vectors, all methods well known and practiced in the art.

In particular, transgenic proteins or viral proteins produced in plant tissues provide a system for expression of peptide epitopes useful as immunogenic peptides in generating vaccines (Usha *et al.* 1993. *Virology* 197:366-374; Mason *et al.* 1993. *Proc. Natl. Acad. Sci. USA* 89:11745-11749). Transgenic plant cells express the genetically introduced foreign peptides which can then be extracted from plant leaves and other plant parts.

An additional method for expressing foreign peptides in plants is to infect plants with plant viruses which have been genetically engineered to express foreign peptides as

antigenic epitopes within modified viral coat protein. The success of such epitope presentation strategies depends on a detailed knowledge of virus structure at the atomic level. Potato virus X (PVX), bean yellow mosaic virus (BYMV), and tobacco mosaic virus (TMV) are examples of well-characterized plant viruses. These viruses are characterized by a single positive-sense RNA genome which is encapsidated by the capsid made up of approximately 2000 copies of a single type of coat protein (CP). For PVX and TMV, proteins which are required for virus replication are translated directly from the genomic RNA; whereas, CP and movement protein (MP), which is involved in cell-to-cell movement, are translated from separate subgenomic mRNAs. The amount of mRNA for the viral proteins determines the amount of each protein produced. The protein produced in the largest amount is the CP, which is as much as 5-10% of the total protein made in the infected plant cell. For BYMV the genomic RNA is translated into a single polyprotein which is cleaved by three virus-encoded proteinases into the mature viral proteins. For each of these viruses, the CP drives the assembly and encapsidation of the viral RNA, which in turn enables long-distance movement and thereby systemic spread of the virus within the plant. Furthermore, the CP is stable, tolerates modification, and exhibits many characteristics of an ideal antigen system.

Foreign peptides can be fused to viral CP and systemically expressed along with the viral CP and in assembled virions. The viruses can be produced at high concentrations; thus, large quantities of the peptide epitope are generated and available for vaccine production. Viruses such as PVX, BYMV, and TMV are candidates for epitope carriers since they are self assembling viruses which aggregate into rod like particles that accumulate in virus infected leaves. The TMV CP, for example, has been shown to be immunogenic (Takamatsu *et al.* 1990. *FEBS. Letts.* 269:73-76) and likely to contain helper T-cell epitopes which could function for chimeric epitopes. Whereas synthetic and recombinant peptides presented to the immune system in a soluble form frequently are poor immunogens and thus require fusion to carriers and formulation in adjuvants to elicit a vigorous immune response, virions carrying repetitive copies of the foreign peptide exposed on the surface serve as potent immunogens. A further advantage of epitope presentation is that coat protein antigens can be isolated and presented in particulate or aggregate form. The particulate nature of TMV based antigens, for example, could be advantageous for maintaining high local concentrations of antigen in parenteral immunizations and may be useful in stimulating mucosal immune responses to orally ingested antigens (Loor, F. 1967. *Virology* 33: 215-220).

Particular regions of the CP are known to be exposed on the surface of the virus particle and to be highly immunodominant (Shukla *et al.* 1989. *Proc. Natl. Acad. Sci. USA* 86: 8192-8196). Therefore, sites of insertion for the foreign peptides are chosen so that the translated foreign peptides are expressed on the surface of the coat protein and thus project outwards on the virus particle. For example, experiments where the 12 amino acid angiotensin-I-converting enzyme inhibitor was fused to tobacco mosaic virus (TMV) coat protein, have suggested that each virus particle contained mostly native coat protein interspersed with subunits composed of the exposed peptide-fusions projected outward (Sugiyama *et al.* 1995. *FEBS Lett.* 359: 247-250). Other examples of successful epitope presentation include malarial epitopes expressed by TMV coat protein (Turpen *et al.* 1995. *Bio-technology* 13: 53-57) and animal virus epitopes expressed by cowpea mosaic virus (CPMV; Lomonossoff *et al.* 1995. *Sem. Virol.* 6: 257-267). Fernández-Fernández *et al.* (1998. *FEBS Letters* 427:229-235) have described an antigen presentation system based on an infectious clone of plum pox potyvirus. Thus, epitope-expressing viruses can be isolated from infected plants and used as epitope presentation vehicles to raise specific antibodies against small peptides.

Antigenic determinant II of Newcastle Disease Virus (NDV) is a continuous epitope consisting of 17 amino acids spanning from Leu_{65} to Leu_{81} of the fusion protein F_0 described by Toyoda *et al.* (1988. *J. Virol.* 62: 4427-4430). This epitope is composed of the amino acids LLPNMPKDKEACAKAPL (SEQ ID NO:12). Monoclonal antibodies specific to antigenic determinant II have been found to be highly potent at neutralizing the infectivity and inhibiting both homolysis and fusion activities of NDV (Abenes *et al.* 1986. *Arch. Virol.* 90: 97-110.) Vaccination of chickens using either a linear plasmid DNA vector expressing the entire F protein (553 amino acids) of NDV (Sakaguchi *et al.* 1996. *Vaccine* 14, 747-752) or a recombinant fowlpox virus expressing the entire F protein of NDV (Taylor *et al.* 1990. *J. Virol.* 64, 1441-1450) gave efficient protection against the disease.

Variability in the human immunodeficiency virus type 1 (HIV-1) gp41 epitope ELDKWA (SEQ ID NO:8) is limited among characterized isolates of HIV-1 making this conserved epitope a candidate for use for vaccine production. The ELDKWA epitope is recognized by the human monoclonal antibody 2F5 which is able to neutralize HIV *in vitro*. Expression of the ELDKWA epitope from a chimeric influenza virus has been shown to induce mucosal immune response in mice, and the antisera induced were able to neutralize multiple strains of HIV-1 *in vitro* (Muster *et al.* 1993. *J. Virol.*

67:6642-6647; Muster *et al.* 1994. *J. Virol.* 68:4031-4034; Muster *et al.* 1995. *J. Virol.* 69: 6678-6686).

The development of *in vitro* expression systems that allow production of infectious positive-sense RNAs from cloned full length cDNA genomes (Dawson *et al.* 1986. *Proc. Natl. Acad. Sci. USA* 83:1832-1836; Meshi *et al.* 1986. *Proc. Natl. Acad. Sci. USA* 83: 5043-5047) has permitted the direct manipulation of the TMV genome at the DNA level. Highly infectious RNA transcripts of a full-length infectious cDNA clone of the U1 (common) strain of TMV have been produced *in vitro* using bacteriophage T7 RNA polymerase (Holt *et al.* 1991. *Virology* 181:109-117) Thus, the RNAs of TMV and other viruses are good candidates as vectors for the expression of foreign genes in plants. However, although TMV, PVX, and other viruses have been used as viral vectors for expressing foreign gene products, such vectors have not always been completely successful. Gene products have been produced via expression from viral vectors; however, the usefulness of such vectors can be limited by instability of inserted sequences and the failure of the viral vector to replicate efficiently. Similarly, there are apparent limitations associated with expression of gene products from genes introduced stably into transgenic plant genomes. Low levels of expression of introduced genes resulting from suppression have been observed and difficulties in recovering functional foreign peptide may be encountered. To obtain high yields of a foreign gene product wherein the peptide of interest is stably expressed and easily purified, it is sometimes advantageous to utilize a strategy involving transencapsidation.

Transencapsidation, or phenotypic mixing, describes the coating of the RNA of one virus or isolate either partially or completely with the CP of another virus or isolate (Rochow 1970. *Science* 167:875-878). Thus, fusion viral coat proteins, which comprise foreign antigenic peptides, and which are expressed in a plant either as a result of Agrobacterium-mediated transfection or as a result of inoculation with infectious viral RNA, can encapsidate the viral genomes of particular related viruses. For example, Farinelli *et al.* (1992. *BioTechnology*10:1020-1025), Lecoq *et al.* (1993. *Mol. Plant-Microbe Interact.* 6:403-406), and Maiss *et al.* (1994. pp.129-139 in: Proc. Int. Symp. Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, 3rd. D.D. Jones, ed. University of California, Division of Agriculture and Natural Resources, Oakland) have demonstrated partial transencapsidation of potyviruses with heterologous CP in transgenic plants.

Bean yellow mosaic virus (BYMV) and potato virus Y (PVY) are members of the potyvirus group. The single genomic RNA is translated into a single polyprotein which is then cleaved by three virus-encoded proteases to yield the mature viral proteins. The viral coat protein (CP) is the C-terminal product on the viral polyprotein, and it is therefore necessary to modify the CP sequence with an initiation codon in a suitable context in order to express the CP as a separate gene in transgenic plants (*e.g.* Hammond and Kamo. 1993. *Acta Hort.* 336:171-178; Hammond and Kamo. 1995. pp.369-389 in: Biotechnology and Plant Protection: Viral Pathogenesis and Disease Resistance. D.D. Bills and S.D. Kung, eds. World Scientific, Singapore.) or from an engineered viral vector. Potyviruses typically have moderate host ranges, but many potyviruses, including both BYMV and PVY, readily infect *Nicotiana benthamiana*, which is readily transformed using *Agrobacterium tumefaciens*. Potyviruses have a CP that is highly conserved at the amino acid level between distinct viruses; the major differences between the CP of distinct viruses, and between isolates of a single virus, are in the N-terminal domain (Shukla *et al.* 1994. The Potyviridae. CAB International, Wallingford, 516pp). The N-terminal domain varies considerably in length between distinct viruses (from about 22 to over 50 amino acids; Hammond, 1992. *Arch. Virol.* [Suppl.5]:123-128), with natural deletions of up to 15 amino acids reported in some isolates.

The similarity of structure between the CP of different potyviruses allows compatibility and mixed assembly of subunits derived from two distinct viruses into a single virion. Such phenotypic mixing or transcapsidation occurs naturally in mixed infections, and can result in complementation leading to aphid transmission of a normally aphid non-transmissible isolate (Hobbs and McLaughlin. 1990. *Phytopathology* 80:268-272; Bourdin and Lecoq. 1991. *Phytopathology* 81:1459-1464). This is also the basis for transcapsidation in potyvirus-infected transgenic plants expressing a potyviral coat protein (Lecoq *et al.* 1993. *Mol. Plant-Microbe Interact.* 6:403-406; Farinelli *et al.* 1992. Bio/Technology 10:1020-1025; Hammond and Dienelt. 1997. *Mol. Plant-Microbe Interact.* 10:1023-1027).

Virus vector systems for epitope presentation are particularly advantageous because such systems ensure that the peptide exposed on the surface can be easily recognized by the immune system of mammals, the virus can be inoculated mechanically to large numbers of plants, very large quantities of purified virus can be recovered using simple extraction from infected leaves within two to four weeks after inoculation, and the resulting viral particles are stable (Scholthof *et al.* 1996. *Annu. Rev. of Phytopathol.* 34: 299-323).

Thus, systemic infection of plants with a virus vector encoding a foreign protein can be an economical means for obtaining unlimited yields of recombinant immunogenic proteins, which can be recovered from the leaves and sometimes other parts of the plant. However, although there have been advances in the art, the need exists for new and improved methods for utilizing positive-sense virus vectors as a means for expressing and producing large quantities of immunogenic foreign proteins *in planta* as well as for new and improved methods for ensuring stability, high yields, and efficient and economical purification of a functional product.

Accordingly, the present invention provides a method for producing in a plant a viral vaccine or an immunogenic peptide, which is very stable, can be economically purified, and is capable of raising an immune response in a mammal.

SUMMARY OF THE INVENTION

We have discovered a method of reproducibly and economically obtaining large quantities of foreign peptide by stably overexpressing non-native, foreign peptides in plants through the insertion of foreign peptide sequences into the viral coat protein of particular plant viruses.

In accordance with this discovery, it is an object of the invention to provide a plant systemically infected and producing stable recombinant plant virions expressing foreign peptides, as a means of producing and purifying large amounts of foreign peptides to be used as genetically engineered vaccines or for antibody production. Plants, plant cells, plant parts, and plant progeny are encompassed in the invention. In particular, a plant in which NDV or HIV immunogenic peptides are expressed is provided.

Another object of the invention is to provide a construct comprising a nucleic acid encoding a fusion viral coat protein comprising a foreign peptide and a truncated bean yellow mosaic virus coat protein (BYMV-CP).

It is an additional object of the invention to provide a host cell containing the nucleic acid of the invention, wherein said host cell is a bacterial cell, in particular, an *Escherichia coli* cell and an *Agrobacterium tumefaciens* cell.

Yet another object of the invention is to provide a method of transforming a plant with a gene capable of expressing a fusion coat protein comprising a foreign peptide and

truncated BYMV-CP. A method of transforming a plant with a gene encoding a fusion coat protein comprising an HIV immunogenic peptide and truncated BYMV-CP is particularly provided.

Still another object of the invention is to provide modified viral vectors encoding fusion coat proteins comprising foreign antigenic peptides. Viral vectors which encode fusion coat proteins which comprise NDV or HIV immunogenic peptides and BYMV-CP are particularly provided.

A further object of the invention is to provide a method of capturing, stably expressing, and purifying fusion coat proteins comprising foreign antigenic peptides by utilizing a transencapsidation strategy. A transencapsidation strategy involving PVY or BYMV virions is particularly provided.

A still further object of the invention is to provide transencapsidated virions incorporating coat proteins comprising foreign antigenic peptides. Transencapsidated virions which encode fusion coat proteins which comprise NDV or HIV immunogenic peptides and truncated BYMV-CP are particularly provided.

Other objects and advantages of this invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-G are schematic diagrams of methods used to produce the BYMV-derived constructs used for expression from PVX and for plant transformation.

Figure 1A shows a linear representation of the BYMV coat protein and the surface-located immuno-dominant epitopes replaced with the vaccine epitope for expression in a transgenic plant or viral vector.

Figure 1B shows the approximate location of PCR primers used to accomplish the removal of the immunodominant BYMV epitopes and replacement with the HIV ELDKWA epitope. The designation for each construct is indicated above the hatched boxes representing the BYMV and HIV epitope-modified CP open reading frames, respectively. BYMV CP modified only with a transcriptional start codon has a predicted molecular mass of 31078 Da. The HIV epitope-modified CP (ELDKWAcp) has a predicted molecular mass of 29710 Da. H: *Hind III*; D: *Dra I*; P: *Pst 1*; B: *Bam H1*; E: *Eco R1*.

Figure 1C depicts the primers JH039, JH040, and JH042, which were used for introduction of the ELDKWA epitope from HIV gp41 into the BYMV CP, in more detail: the forward primer JH039: 5'-GAAGGAAATCCTAATGAGCTCGATAAGTGGGCAAGT-GTCAGGCAAATAGTACC-3' (SEQ ID NO:1); the reverse primer JH040: 5'-CTTTTT-CCTTTTATCGAGCTCATTTGACCATGCATTGAGTTGCTCTTGATCTGC-3' (SEQ ID NO:2) which is the complement of 5'-GCAGATCAAGAGCAACTCAATGCATGGTCA-AATGAGCTCGATAAAAGGAAAAAG-3' (SEQ ID NO:3); and forward primer JH042: 5'-GATTACGCCAAGCTTTAAAACAATGGCAGATCAAGAGCAACTCAATGC-3' (SEQ ID NO:4). (1) Upper lines: The N-terminal portion of the BYMV CP sequence (SEQ ID NO:6) and translation (one letter code; SEQ ID NO:7). Lower lines: Primer JH039 sequence (SEQ ID NO:1) and translation (one letter code; SEQ ID NO: 8). Only those amino acids contributing to the ELDKWA epitope are shown. (2) Upper lines: BYMV sequence (SEQ ID NO:6) and translation (one letter code; SEQ ID NO:7). Lower lines: Complement (SEQ ID NO:3) of Primer JH040 sequence (SEQ ID NO:2) and translation (one letter code; SEQ ID NO: 9). Only those amino acids contributing to the ELDKWA epitope are shown. (3) Primer JH042 sequence (SEQ ID NO:4) and translation (one letter code; SEQ ID NO: 5).

Figure 1D is a diagram indicating the particular amino acids of the highly hydrophilic epitopes (BOLDFACE type) which were removed in the native BYMV CP, and subsequently replaced with the ELDKWA-HIV epitope (BOLDFACE type) to generate the amino terminal sequence (SEQ ID NO:10) and translation (one letter code; SEQ ID NO:11) of the ELDKWA epitope-modified BYMV CP. The nucleic acid sequence of the N-terminal portion of the BYMV CP sequence, 5'-ATGGCAGATCAAGAGCAACTCAAT-GCAGGTGAGGAGAAGAAGGATAAAAGGAAAAAGAATGAAGGAAATCCTAATAAG GACTCTGAGGGGCAGAGTGTCAGGCAAATAGTACC-3', is identified by SEQ ID NO:6; the one letter code translation, MADQEQLNAGEEKKDKRKKNEGNPNKDSEGQSVR-QIV, is identified by SEQ ID NO:7.

Figure 1E shows the deliberate primer-dimer of JH042/JH040 used to create the 5' portion of ELDKWAcp, which was then annealed, extended, and amplified by PCR. Upper lines: Sequence (SEQ ID NO:4) and translation (one letter code; SEQ ID NO: 5) of primer JH042; underlined is the *Hind III* site used in subsequent subcloning into pGA643. The M in the one letter code indicates the initiation codon for plant

expression. Lower lines: Sequence (SEQ ID NO:3) and translation (one letter code; SEQ ID NO:9) of the complement of primer JH040; underlined is the *Sac I* site used for assembly with the 3' portion of ELDKWAcp. Only those amino acids contributing to the ELDKWA epitope are indicated.

Figure 1F and 1G are schematic diagrams of intermediates in the construction of the ELDKWA cp. Figure 1F shows the construction of pCR2.1.JH042/JH040, including removal of the H:H fragment to remove unwanted *Sac1*, *Bam H1*, and *Eco R1* sites from the vector. SP6: SP6 promoter; H: *Hind III*; S: *Sac I*; B: *BamH1*; E: *EcoRI*; X: *Xba I*.

Figure 1G shows the construction of pCR2.1.JH039/M13F. The deliberate primer-dimer of JH042/JH040, and the PCR product JH039/M13F were separately cloned into the vector pCR2.1. The plasmids were screened for the appropriate inserts, which were subsequently ligated together at the introduced *Sac I* site to create pCR2.1.ELDKWAcp (See Figure 1B). SP6: SP6 promoter; H: *Hind III*; S: *Sac I*; B: *BamH1*; E: *EcoRI*; X: *Xba I*.

Figure 2 is a representation of the nucleotide and amino acid sequence of the entire modified BYMV CP containing the NDV F protein epitope, LLPNMPKDKEACAKAPL (SEQ ID NO:12). The location of the forward primer NDV1 (5'-CCCAAGCTTAATTAA-TACAATGGCAGATCAAGAGCAATTGTTGCC-3'; SEQ ID NO:13), the reverse primer NDV2 (5'-TTTGCGCATGCTTCCTTATCCTTTGGCATATTTGGCAACAATTGCTCTTG-3'; SEQ ID NO:14), the forward primer BYMCP1(5'-GGAAGCATGCGCAAAGGCACC-ATTGGTCAGGCAAATAGTACCA-3'; SEQ ID NO:15), and the reverse primer BYMVCP2 (5'-GGAATTCTCGAGCTAAATACGAACACCAAGCA-3'; SEQ ID NO:16) used in the construction of the BYMVF CP construct, identified by SEQ ID NO:17, is shown. The complement of SEQ ID NO:17 is identified by SEQ ID NO:18. SEQ ID NO:19 identifies the one letter code translation of SEQ ID NO:17.

Figure 3 is a Western analysis of triplicate blots of identical samples derived from virus infected plants. Purified virus obtained from infected plants were electrophoresed on 10% polyacrylamide/SDS gels. The samples lanes contained: PVY, purified potato virus Y; BYMV, purified bean yellow mosaic virus; TMVF, potato virus Y purified from plants co-infected with the TMV vector expressing the BYMVF CP (NDV-F epitope on the BYMV coat protein (CP)); PVXF, potato virus Y purified from plants co-infected with the PVX vector expressing the BYMVF CP. The separated protein was

transferred to Immobilon membranes. Following transfer, the blots were reacted to three different types of antisera. The blots were then developed with NBT/BCIP. The blots were reacted with the following antisera, as noted on the figure: Figure 3A, PTY2, Monoclonal antibody that reacts with all potyviruses (here PVY and BYMV); Figure 3B, PTY3, Monoclonal antibody that reacts with BYMV and not PVY; Figure 3C, NDV2190, Polyclonal antibody that reacts only with NDV.

Figure 4 shows Western blots of purified potyvirus preparations of BYMV isolate Ideal A from non-transgenic plants of *Nicotiana benthamiana*, and transgenic lines of *N. benthamiana* expressing the HIV epitope-modified ELDKWAcp. Preparations were diluted to approximately 1mg/ml, dissociated and electrophoresed as described (Hammond and Lawson, 1988, J. Virol. Methods 20:203-217), and blotted to Immobilon PVDF membrane. Following transfer, the blots were reacted with three different types of antibody: Figure 4A, with a mix of potyvirus cross-reactive MAbs (PTY 1, PTY 2, PTY 3, PTY 4, PTY 8, PTY 10, and PTY 21); Figure 4B, with BYMV-specific MAb PTY 24; and Figure 4C, with HIV ELDKWA epitope-specific human MAb 2F5. In each of Figures 4A, 4B, and 4C, the lanes are: Lane 1, Non-transgenic (BYMV-Ideal A, purified from non-transgenic plants); Lane 2, ELDKWA 40-7 (BYMV-Ideal A, purified from transgenic line ELDKWA 40-7, expressing ELDKWAcp); Lane 3, ELDKWA 42A-7 (BYMV-Ideal A, purified from transgenic line ELDKWA 42A-7); Lane 4, ELDKWA 30-4 (BYMV-Ideal A, purified from transgenic line ELDKWA 30-4); Lane 5, ELDKWA 76B-6 (BYMV-Ideal A purified from transgenic line ELDKWA 76B-6); Lane 6, ELDKWA 6-4 (BYMV-Ideal A, purified from transgenic line ELDKWA 6-4). Each transgenic line represents a separate homozygous line from a distinct transformation event. MAb 2F5 reacts only with the virus preparations from transgenic plants, and with a band (open arrowhead to right) migrating slightly faster than the wild-type BYMV-CP (solid arrow head) that forms the major reactive bands in Figures 4A and B.

Figure 5 shows an immunoelectron micrograph of purified PVY virions from plants co-inoculated with PVX-containing the BYMV-NDV coat protein and PVY; the small black dots (gold particles) denote a reaction of the NDV antibody to the surface of the virion, indicating the presence of the NDV epitope.

Figures 6A-6F show results of an indirect enzyme-linked immunosorbent assay (ELISA) demonstrating the incorporation of ELDKWAcp expressed in transgenic plants into virions of BYMV-Ideal A purified from BYMV-inoculated plants. Serial two-fold dilutions of virus preparations, starting at 5µg/ml, were coated directly to ELISA

plates, and subsequently reacted with a mix of PTY monoclonal antibodies (cross-reactive; Figures 6A and 6B), with BYMV-specific PTY 24 (Figures 6C and 6D), or with HIV ELDKWA epitope-specific human neutralizing MAb 2F5 (Figures 6E and 6F). The BYMV preparations from ELDKWA transgenic plants are: △, ELDKWA 40-7; □, ELDKWA 42A-7; ▽, ELDKWA 30-4; and ◇, ELDKWA 76B-6; (○), non-transgenic plants.

Figure 7 shows the Western blot analysis of NDV epitopes using mouse serum from mice injected with purified PVY virions bearing the F epitope of NDV. Purified recombinant TMV carrying the F epitope on the BYMV CP (Left lane) or inactivated NDV were electrophoresed on a 10% SDS polyacrylamide gel. The proteins were transferred to an Immobilon membrane which was subsequently incubated with serum derived from mice that had been injected with purified PVY virions bearing the NDV F epitope. The arrow points to the location of the 58,000 Da F protein in inactivated NDV isolated from diseased chickens.

Figure 8 shows the bacterial expression of the HIV epitope-modified BYMV CP (ELDKWAcp), and specific recognition of the ELDKWA epitope by the HIV-specific neutralizing human monoclonal antibody (MAb) 2F5. Extracts of *Escherichia coli* strain DH5α, and purified BYMV (strain Ideal A) were electrophoresed through a 10% polyacrylamide gel, and blotted to an Immobilon PVDF membrane. Identical sets of lanes were reacted with BYMV isolate GDD- (BYMV-GDD-) specific MAb PTY 43 which reacts with the N-terminal portion of the BYMV-GDD CP, with potyvirus cross-reactive MAb PTY 2, reactive with the core region of the CP, and with ELDKWA epitope-specific MAb 2F5. In each panel, the lanes are as follows: BYMV-Ideal A (Purified BYMV, isolate Ideal A); DH5α/pCR2.1 (*E. coli* strain DH5α carrying plasmid vector pCR2.1 - negative control #1); DH5α/ELDKWAcp (*E. coli* strain DH5α carrying plasmid pCR2.1/ELDKWAcp, expressing the ELDKWAcp as a *lacZ* fusion protein); DH5α/BYMV-CP (*E. coli* strain DH5α carrying plasmid pBY9, expressing BYMV-GDD CP as a *lacZ* fusion protein); and DH5α (without any plasmid - negative control #2). This figure also demonstrates the lack of reactivity of ELDKWAcp with the BYMV isolate GDD-specific monoclonal antibody PTY 43, as a result of the replacement of the N-terminal BYMV epitope (recognized by PTY 43) with the ELDKWA epitope.

Figures 9A-9D show the expression of ELDKWAcp from the PVX vector inoculated to non-transgenic plants of *Nicotiana benthamiana*, as demonstrated by reactivity with potyvirus cross-reactive, BYMV-specific, and ELDKWA-specific MAbs in an indirect ELISA. ELISA plates were coated with extracts of plants, blocked, and incubated with

the appropriate antibody solution (Figure 9A, PVX-specific rabbit polyclonal; Figure 9B, PTY 1 potyvirus cross-reactive mouse MAb; Figure 9C, PTY 24 BYMV-specific mouse MAb; and Figure 9D, 2F5 HIV-specific human MAb) . Plates were again washed prior to addition of goat anti-rabbit (for detection of PVX-specific polyclonal antiserum), goat anti-mouse (for PTY 1 and PTY 24), or goat anti-human (for MAb 2F5) alkaline phosphatase conjugate. Samples of plant extracts were: A, healthy *Nicotiana benthamiana* (negative control); B, BYMV-infected *Nicotiana benthamiana* (BYMV control); C, *Nicotiana benthamiana* plant 1A infected with PVX carrying the ELDKWAcp as an additional gene; and D, *Nicotiana benthamiana* plant 6A infected with PVX carrying the ELDKWAcp as an additional gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to modified viral vectors encoding fusion coat proteins comprising foreign antigenic peptides. The recombinant viral RNA provides for systemic infection of a plant and the resulting production of stable recombinant plant virions expressing foreign peptides, as a means of producing and purifying large amounts of foreign peptides to be used as genetically engineered vaccines or for antibody production. Constructs of the invention encode a fusion protein comprising foreign peptide and a truncated potyvirus CP. The recombinant construct of the invention can be used to produce transgenic plants which express the fusion coat protein comprising a foreign peptide and a truncated potyvirus CP or to generate viral vectors which are genetically engineered to comprise the nucleic acid encoding the fusion protein comprising a foreign peptide and a truncated potyvirus CP. Constructs, viral vectors, and transgenic plants comprising nucleic acid encoding the fusion protein comprising a foreign peptide and a truncated BYMV CP are particularly provided. The genetically engineered viral vectors of the invention can be transcribed and the resultant infectious RNA used to inoculate plants to generate virions expressing a fusion protein comprising a foreign peptide and a truncated potyvirus CP. RNA used to inoculate plants to generate virions expressing a fusion protein comprising a foreign peptide and a truncated BYMV CP is particularly provided. In plants that have been co-infected with a compatible potyvirus, transencapsidation can occur yielding large quantities of virions which stably incorporate a fusion protein comprising a foreign peptide and a truncated potyvirus CP. Such virions can be purified efficiently, economically, and in large quantities. Transencapsidated virions which incorporate fusion coat proteins which comprise NDV or HIV immunogenic peptides and truncated BYMV CP are particularly provided.

The present invention provides a construct encoding a recombinant BYMV fusion CP comprising the amino acid sequence for a foreign protein, preferably of between 5 and 20 amino acids, and a truncated BYMV coat protein. A PVX vector, pP2C2S, was genetically engineered to contain the construct encoding the fusion protein comprising the foreign peptide and the truncated BYMV CP. Specifically, pP2C2S was engineered to contain the construct FCP encoding a NDV epitope (F) and the truncated BYMV CP (CP); pP2C2S was separately engineered to contain the construct ELDKWAcp encoding an HIV epitope "ELDKWA" (SEQ ID NO:8) and the truncated BYMV CP (ELDKWAcp). The vector pPVX-FCP and pPVX-ELDKWAcp has two types of promoters. Transcription by T7 polymerase of the cloned cDNA encompassing the full genome, operably linked to the promoter of the T7 polymerase gene, produces a full length transcript. This transcript, when inoculated into suitable plant species, such as tobacco plants, causes infection and is therefore referred to herein as an "infectious clone" of the virus. The PVX vector also contains a subgenomic promoter which has been duplicated. One copy of the subgenomic promoter is used to make PVX coat protein. The other copy can be used to make any other protein of interest. For example, the vector can be engineered to promote a foreign peptide such as a NDV epitope or an HIV epitope. The modified infectious cDNA clone so produced encodes a PVX MP and a PVX CP. It also encodes a modified CP, *i.e.*, the FCP or ELDKWAcp as described above. Both the wild type PVX coat protein and the coat protein FCP (or ELDKWAcp) are expressed in the plant; however, only wild-type PVX virions (PVX genome assembled with PVX CP) will be assembled.

Thus, as a result of inoculation of the plant with the infectious modified viral PVX RNA, modified BYMV coat proteins expressing foreign peptide are expressed throughout the plant. Furthermore, in plants that have been previously infected with PVY or BYMV virions, transencapsidation will occur as potyvirions are assembled. The PVY (or BYMV) coat protein is incorporated into the viral capsid of the potyvirions along with the modified CP expressed from the PVX vector, thereby assuring formation of a stable capsid that incorporates a substantial proportion of the modified CP containing the foreign antigenic peptide. Virions are generated which express the foreign antigenic peptide, FCP, in the capsid together with PVY (or BYMV) CP. Systemic infection results in the generation of large numbers of virions which express the foreign antigenic peptide. Hence, viral purification yields large quantities of foreign antigenic peptide.

One skilled in the art will appreciate that the modified infectious clone can also be further modified to replace the T7 polymerase promoter by any other strong promoter for transcription of the cDNA clone *in vitro* by contact with a polymerase therefore; or by replacement with a suitable plant promoter for transcription *in vivo* following inoculation of plants with plasmid DNA. Such modified clones are also contemplated within the scope of this invention.

Any antigenic epitope of from about 5 to 20 amino acids can be used for which antibodies are known or are discovered that neutralize a virus or other pathogen, as shown by routine tests well known in the art. For instance, antigenic determinant II of NDV and the antigenic epitope ELDKWA from gp 41 of the HIV virus can be successfully incorporated into the truncated CP of BYMV, and subsequently into transencapsidated BYMV or PVY virions. Furthermore, variants of antigenic epitopes, including antigenic determinant II of NDV and the antigenic epitope ELDKWA, can be used. A "variant" antigenic epitope may have an amino acid sequence that is different by one or more amino acid "substitutions". The variant may have "conservative substitutions", wherein a substituted amino acid has similar structural or chemical properties, *e.g.,* replacement of leucine with isoleucine. More rarely, a variant may have "nonconservative" changes, *e.g.*, replacement of a glycine with a tryptophan. Similar minor variations may also include amino acid deletions or insertions, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTAR software. "Immunological activity" defines the capability of the recombinant variant epitope, or any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies. The recombinant nucleic acid molecules encoding the variant epitopes are encompassed by the invention as are virions which are generated to express variants of the foreign antigenic peptide.

As used herein a suitable plant host is any variety of plant known to be subject to infection by PVX, BYMV, PVY, and TMV. For instance suitable host plants for TMV include lettuce, spinach, tomato, potato as well as *Nicotiana tabacum, N. glutinosa, N. sylvestris, N. benthamiana, Phaseolus vulgaris* and *Chenopodium amaranticolor.*

Since the modified CP is produced under the control of the virus promoter rather than under the control of the plant promoter, up to 10 percent of the total protein in the plant is the modified CP containing the FCP of this invention.

Therefore, in one embodiment, transencapsidation involving viral transcripts of the CP-modified PVX infectious clone and infectious PVY virions is used to accomplish systemic infections of suitable host plants. After construction of the modified clones, the PVX DNA constructs are transcribed *in vitro* by suitable reactions carried out in the presence of a polymerase active with the promoter (*i.e.*, T7 polymerase with the T7 promoter) to generate RNA for inoculation of PVY-infected plants.

In another embodiment, the modified CP comprising the foreign antigenic peptide is provided by using a transgenic host plant wherein the transgene encodes a fusion protein comprising truncated BYMV CP and the HIV-I gp41 ELDKWA epitope. Transcription of the transgene and translation yields copies of the modified CP, *i.e.*, the truncated BYMV CP which contains the HIV-I epitope. Infection of this transgenic plant with wild type BYMV infectious virions results in viral replication of the wild type BYMV within the transgenic host plant. During viral replication and assembly of wild type BYMV virions, a portion of the virions isolated from the plants will be transencapsidated with the transgene CP. Thus, the BYMV virions will contain foreign HIV-I epitopes. The plant undergoes systemic infection of the virus, resulting in transencapsidation in all infected tissues.

The method of exposure can be by inoculation of the subject with purified antigen at appropriately spaced intervals using methods routine in the art or by the subject ingesting plants or plant tissue extracts that have been infected with the virions produced from the CP-modified clones of this invention into which a nucleotide sequence encoding a heterologous viral or other antigen has been encoded. The purified virus or a plant in which the recombinant virus is accumulated is administered in an immune response stimulating dose as determined by those skilled in the art taking into account, for instance, the body weight and general health of the subject.

Modified PVY or BYMV virions produced by transencapsidation have the heterologous viral or other antigen projecting from the surface of the viral coat, and are capable of stimulating producing of antibodies in the subject that neutralize the antigen. Alternatively, immunogenic exposure can be by a combination of inoculation and ingestion of the antigenic epitopes, or by ingestion alone. The virus can be ingested directly by intubation or oral inoculation of a subject can be accomplished by the subject consuming a sufficient quantity of a plant, such as spinach, that has been systemically infected with a CP-modified infectious clone to raise a neutralizing level

of antibodies to the heterologous antigenic epitope encoded by the CP-modified infectious clone.

Virus can also be grown and maintained in protoplasts of plant lines such as *Nicotiana benthamiana*. Generally *in vitro* transcripts are inoculated into tobacco protoplasts by electroporation as described by Watanabe *et al.* (1987. *FEBS Lett.* 219:65-69). In addition, virus can be grown in cultures of infected cells. Such cells can be maintained for extended periods of time (Murakishi *et al.* 1971. *Virology* 43:62-68).

One skilled in the art will appreciate that antigenic peptides and/or virions can be purified from plant leaves using standard methods (Bruening *et al.* 1976. *Virology* 71: 498-517). Generally virus is purified from infected leaf tissues by homogenizing the infected leaf tissues in appropriate buffer, removing the leaf debris, and concentrating the virus either by a salting out procedure or by ultracentrifugation. The standard methods for purification of TMV, for example, can lead to the isolation of 1 to 5 mg of purified virus per gram fresh weight of tobacco leaves.

EXAMPLES

Having now generally described this invention, the following examples illustrate the manner in which the invention can be practiced. It is understood, however, that the xamples are included herein only to further illustrate the invention and are not intended to limit the scope of the invention as defined by the claims.

EXAMPLE 1

BYMV-Foreign Epitope Constructs

The immunodominant surface epitopes of bean yellow mosaic virus coat protein were replaced with a vaccine epitope for subsequent expression in a transgenic plant or viral vector (Figure 1A). The forward primer JH039 (5'-GAAGGAAATCCTAATGAGCTCGAT-AAGTGGGCAAGTGTCAGGCAAATAGTACC-3') (SEQ ID NO:1), the reverse primer JH040, 5'-CTTTTTCCTTTTATCGAGCTCATTTGACCATGCATTGAGTTGCTCTTG-ATCTGC-3' (SEQ ID NO:2) which is the complement of 5'-GCAGATCAAGAGCAACTC-AATGCATGGTCAAATGAGCTCGATAAAAGGAAAAAG-3' (SEQ ID NO:3), and forward primer JH042, 5'-GATTACGCCAAGCTTTAAAACAATGGCAGATCAAGAGCAACTC-AATGC-3' (SEQ ID NO:4) were used to accomplish the removal of the immunodominant BYMV epitopes and replacement with the HIV ELDKWA epitope (Figures 1B). BYMV CP modified only with a transcriptional start codon has a predicted molecular mass of

31078 Da. The HIV epitope-modified CP (ELDKWAcp) has a predicted molecular mass of 29710 Da (Figure 1B). The C-terminal portion of ELDKWAcp is identical to that of the FCP construct shown in Figure 2. The primers are described in more detail in Figure 1C. The particular amino acids of the highly hydrophilic epitopes which were removed in the native BYMV CP, and subsequently replaced with the ELDKWA-HIV epitope are shown in Figure 1D . A deliberate primer-dimer of JH042/JH040 was used to create the 5' portion of ELDKWAcp which was then annealed, extended, and amplified by PCR (Figure 1E). The deliberate primer-dimer of JH042/JH040, and the PCR product JH039/M13F were separately cloned into the vector pCR2.1. The plasmids were screened for the appropriate inserts, which were subsequently ligated together at the introduced *Sac I* site. Figure 1F and Figure 1G are schematic diagrams of intermediates in the construction of the ELDKWAcp; Figure 1B shows the construction of pCR2.1 ELDKWAcp containing the ELDKWA peptide at the 5' terminus as a fusion protein.

EXAMPLE 2

Plasmid Construction to Produce Chimeric Virus Vectors

Newcastle Disease Virus (NDV)

Antigenic determinant II of Newcastle Disease Virus (NDV) fusion protein (F) is a continuous epitope which consists of 17 amino acids spanning from Leu_{65} to Leu_{81} of F_0 (Toyoda *et al.* 1988. *J. Virol.* 62: 4427-4430). A DNA fragment encoding the major portion of the epitope was PCR amplified with a pair of partially complementary oligonucleotide primers NDV1 and NDV2. The location of the forward primer NDV1 (5'-CCCAAGCTTAATTAATACAATGGCAGATCAAGAGCAATTGTTGCC-3'; SEQ ID NO:13) and the reverse primer NDV2 (5'-TTTGCGCATGCTTCCTTATCCTTTGGCATA-TTTGGCAACAATTGCTCTTG-3'; SEQ ID NO:14) are shown in Figure 2. Specific sequence tags were engineered into the primers so that the amplified epitope-encoding fragment contains a *Hind* III recognition site as well as a translation initiation codon at the 5' end and a *Sph*I recognition site at the 3' end. A truncated BYMV CP gene lacking the first 40 codons was amplified from plasmid pBY9 (Hammond and Hammond. 1989. *J. Gen. Virol.* 70: 1961-1974) with primer pair BYMVCP1/BYMVCP2. The location of the forward primer BYMCP1 (5'-GGAAGCATGCGCAAAGGCACCATTGGTCAGG-CAAATAGTACCA-3'; SEQ ID NO:15) and the reverse primer BYMVCP2 (5'-GGAATT-CTCGAGCTAAATACGAACACCAAGCA-3'; SEQ ID NO:16) are also shown in Figure 2. Primer BYMVCP1 was tagged with an *Sph*I recognition site and a sequence encoding the last 5 amino acids of the F epitope, overlapping primer NDV2. *Eco*RI and *Xho*I recognition sites were engineered at the end of primer BYMVCP2. The amplified epitope-encoding fragment and the truncated BYMV-CP gene were digested

with *Sph*I and ligated to form an NDV-F/BYMV-CP chimera. This chimera was in turn inserted into plasmid vector pUC19 at *Hin*dIII/*Eco*RI sites giving rise to pFCP. The resulting construct is shown in Figure 2 and is identified by SEQ ID NO:17.

To create pPVX-FCP, a PVX (potato virus X)-based vector pP2C2S was digested with *EcoRV* and dephosphorylated with CIP. The NDV-F/BYMV-CP insert was prepared from pFCP by *Hin*dIII/*Eco*RI double digestion followed by Klenow fragment filling-in. After ligation of the insert into the pP2C2S vector, a recombinant with the desired orientation was selected.

To create pTMV-FCP, a TMV-based vector p30BRz was digested with *Pin*AI, filled-in with Klenow fragment of *E.coli* DNA polymerase I, and digested with *Xho*I. The NDV-F/BYMV-CP insert was prepared from pFCP by *Hin*dIII digestion and Klenow fragment filling-in followed by *Xho*I digestion. The above-treated vector and insert were ligated with T4 DNA ligase.

Human Immunodeficiency Virus Type I (HIV)

Two approaches were used to express a unique HIV epitope as part of a plant viral capsid protein. The first involved the transformation of *Nicotiana benthamiana* with *Agrobacterium tumefaciens* containing a binary plasmid in which a bean yellow mosaic virus (BYMV) coat protein gene where 22 amino acids were replaced by 9 amino acid residues of the HIV gp41 epitope (amino acids ELDKWA) was engineered (Figures 1B, 1F, and 1G). The ELDKWAcp construct was transferred as a *HindIII/BamH1* fragment into pGA643 digested with *Hin*dIII and *Bgl* II, creating pGA/ELDKWAcp. The pGA/ELDKWAcp was transformed into *Agrobacterium tumefaciens* strain C58C1, and used to transform leaf pieces of *N. benthamiana*. Plants were transformed and selected essentially as described by Hammond and Kamo (1995. Mol. Plant-Microbe Interact. 8:674-682). Transformed plants expressing detectable ELDKWAcp were identified by antigen-coated plate indirect ELISA with potyvirus cross-reactive MAbs (Jordan and Hammond. 1991. *J. Gen. Virol.* 72:25-36) and ELDKWA-specific human MAb 2F5 (Muster *et al.* 1993. *J. Virol.* 67:6642-6647). The plants expressing the modified CP were then inoculated with wild type BYMV and a portion of the virions isolated from infected plants were transencapsidated with the ELDKWAcp (See Example 4, below).

The second approach was similar to that described above for the NDV epitope. The engineered CP gene was excised from pCR2.1(ELDKWAcp) with *Dra I,* which cleaves immediately 5' to the initiation codon, and 3' to the termination codon of the

ELDKWAcp (Figure 1B), and inserted at the *Eco* RV site of pSKAS (immediately downstream of the PVX subgenomic promoter), creating pSKAS/ELDKWAcp. The *Apa* 1/*Spe* 1 fragment from pSKAS/ELDKWAcp was then inserted into the equivalent sites of the full-length PVX viral-based vector pP2C2S and infectious transcripts were prepared and delivered to plants as outlined below for the NDV-epitope containing CP.

EXAMPLE 3

Preparation and Delivery of Infectious Transcripts

Both the PVX-based vector pP2C2S and the TMV-based vector p30BRz contain a bacteriophage T7 promoter prior to the respective viral template, facilitating the production of infectious viral transcripts *in vitro* using T7 RNA polymerase-driven transcription. For the PVX-based construct pPVX-FCP, the plasmid DNA was linearized with *Spe*I prior to transcription. For TMV-based construct pTMV-FCP, since the vector contains an engineered cis-acting ribozyme at the 3' terminus of the viral template, no template linearization is necessary before transcription. Approximately one µg of each DNA template was used in a 20 µl reaction for synthesis of capped transcripts using T7 Message Machine kit (Ambion) at 37°C. Considering the size of the expected transcripts, 1 µl of 30 mM GTP was supplemented 15 min after the start of the reaction. The transcription reaction was continued at 37°C for 90 min. An aliquot of the product (2 µl) was fractionated on a 1.0% agarose gel to assess the quantity and the integrity of the transcripts. The transcripts were diluted to 0.5 µg/µl with 50 mM potassium phosphate (pH 7.0) for inoculation.

The production of vaccines in our system is based on the observation that potyvirus capsid proteins are capable of encapsidating heterologous potyvirus RNAs and that progeny virions in some cases may contain up to 25% of the second coat protein (Hammond and Dienelt. 1997. *Mol. Plant Microbe Interact.* 10: 1023-1027). Young healthy *Nicotiana tabacum* seedlings were first inoculated with potato virus Y (PVY)

tissue in 1% K_2HPO_4 at approximately 1g leaf/10ml with a mortar and pestle in the presence of a small amount of Celite, and manually inoculating three to four leaves of plants with a finger dipped in the inoculum. The virus purification procedure was essentially the same as described by Hammond and Lawson (1988. *J. Virol. Meth.* 20: 203-217). Briefly, leaves were homogenized in 5 volumes (w/v) of 0.5M potassium phosphate buffer (pH 8.4) supplemented with 0.5% Na_2SO_3. The homogenate was filtered through two layers of cheesecloth and the filtrate was centrifuged at 4500 rpm (Sorvall GSA) for 10 min at 4°C. Crude virus particles were precipitated from the supernatant with the addition of PEG 8000 to a final concentration of 4% in the presence of 0.1 M NaCl. After centrifugation at 7000 rpm (Sorvall GSA, 4°C, 10 min), the pellet was collected and resuspended in 0.1M borate/KCl buffer (8.0). The virus particles were dispersed and concentrated by centrifugation through a 30% sucrose pad (27,000 rpm, 2.5 hrs, Beckman R30), resuspended again in 0.1M borate/KCl buffer (8.0) and finally purified through CsCl gradient centrifugation at 40,000 rpm (Beckman R65) for 16 hrs at 10°C, followed by dialysis to remove CsCl.

EXAMPLE 5

Immunoblot Analysis of Transencapsidated Virions

For the NDV epitope experiments, three µg of viral proteins as determined by Bradford assay were separated by 12% SDS-PAGE and transferred to an Immobilon-P membrane (Millipore). The transferred proteins were probed with mouse anti-potyvirus CP monoclonal antibodies PTY 2, PTY 3, or PTY 24, or a mix of potyvirus cross-reactive MAbs (PTY 1, PTY 2, PTY 3, PTY 4, PTY 8, PTY 10, and PTY 21) and separately with the chicken anti-NDV polyclonal antibody '2-1-90' (NDV2190; obtained from Jack King, USDA-ARS) followed by alkaline phosphatase (AP)-conjugated goat anti-mouse, goat anti-chicken, or goat anti-human antibodies (Kirkegaard & Parry Laboratories, Inc.) as appropriate. The immunoreactions were visualized by the addition of AP substrate BCIP-NBT.

The samples lanes of the experiment shown in Figure 3 contain: PVY, purified potato virus Y; BYMV, purified bean yellow mosaic virus; TMVF, potato virus Y purified from plants co-infected with the TMV vector expressing the BYMVF CP (NDV-F epitope on the BYMV coat protein (CP)); PVXF, potato virus Y purified from plants co-infected with the PVX vector expressing the BYMVF CP. The blots were reacted with the following antisera, as noted on the figure: PTY2, monoclonal antibody that reacts with all potyviruses (here PVY and BYMV); PTY3, monoclonal antibody that reacts with BYMV and not PVY; NDV2190, polyclonal antibody that reacts only with NDV.

The results are as follows. In Figure 3A, all samples react with PTY2, indicating that all contain potyvirus coat proteins, as expected, for the TMV and PVX samples are from plants co-infected with PVY and the BYMV CP should be also expressed. In Figure 3B, only three samples react with PTY3, which is specific for BYMV. The results demonstrate that BYMV CP is being encapsidated on the purified PVY particles in TMVF and PVXF. In Figure 3C, NDV2190 only reacts with TMVF and PVXF, showing that the BYMVF CP that is expressed from both of these vectors is encapsidated in the PVY particle and the F epitope of NDV is expressed on the particle in such a way that it is recognized by the antiserum to NDV.

For the HIV epitope experiments, 15 ul of approximately 1mg/ml purified virus preparations as determined by UV absorption were separated by 10% SDS-PAGE. Purified virus preparations of BYMV isolate Ideal A from non-transgenic plants of *Nicotiana benthamiana*, and transgenic lines of *N. benthamiana* expressing the HIV epitope-modified ELDKWAcp were diluted to approximately 1mg/ml, dissociated and electrophoresed as described (Hammond and Lawson. 1988. *J. Virol. Methods* 20:203-217), and blotted to Immobilon PVDF membrane (Millipore). The purified viral preparations were: BYMV-Ideal A, purified from non-transgenic plants; ELDKWA 40-7 - BYMV-Ideal A, purified from transgenic line ELDKWA 40-7, expressing ELDKWAcp; ELDKWA 42A-7 - BYMV-Ideal A, purified from transgenic line ELDKWA 42A-7; ELDKWA 30-4 - BYMV-Ideal A, purified from transgenic line ELDKWA 30-4; ELDKWA 76B-6 - BYMV-Ideal A, purified from transgenic line ELDKWA 76B-6; ELDKWA 6-4 - BYMV-Ideal A, purified from transgenic line ELDKWA 6-4. Each transgenic line represents a separate homozygous line from a distinct transformation event. The transferred proteins were probed with mouse anti-potyvirus CP monoclonal antibodies: PTY 24 , a mix of potyvirus cross-reactive MAbs (PTY 1, PTY 2, PTY 3, PTY 4, PTY 8, PTY 10, and PTY 21; Jordan and Hammond, *supra*), or with human monoclonal antibody 2F5 specific for the ELDKWA epitope (obtained from H. Katinger, Austria; Muster *et al*, 1993, *supra*) followed by alkaline phosphatase (AP)-conjugated goat anti-mouse, goat anti-chicken, or goat anti-human antibodies (Kirkegaard & Parry Laboratories, Inc.) as appropriate. The immunoreactions were visualized by the addition of AP substrate BCIP-NBT.

ELDKWAcp, expressed from the transgenic plants, is incorporated into potyvirus virions formed in plants challenged with BYMV (transcapsidation) as indicated by reactivity of HIV epitope ELDKWA-specific MAb 2F5 with virus preparations purified from transgenic plants expressing the epitope-modified ELDKWAcp (Figure 4C). All virus preparations reacted with the mix of potyvirus cross-reactive MAbs (Figure 4A)

and with the BYMV-specific MAb PTY 24 (Figure 4B). MAb 2F5 reacted only with the virus preparations from transgenic plants, and with a band (open arrowhead to right) migrating slightly faster than the wild-type BYMV-CP (solid arrow head) that forms the major reactive bands in Figures 4A and 4B (Figure 4C). A faint band corresponding to ELDKWAcp is reactive with the "PTY mix" (Figure 4A) and PTY 24 (Figure 4B).

EXAMPLE 6

Immunoelectron Microscopy

Purified virions were analyzed by electron microscopy following labeling with antibodies specific for potyviruses, NDV, and/or ELDKWA, and by ELISA with appropriate antibodies, essentially as reported (Hammond and Dienelt. 1997. *Mol. Plant Microbe Interact.* 10: 1023-1027). The results provide evidence for the transencapsidation fo the chimeric BYMV coat proteins into infectious virus particles of PVY (Figure 5).

EXAMPLE 7

Enzyme-linked Immunosorbent Assay (ELISA)

Purified virions were analyzed by ELISA following labeling with antibodies specific for potyviruses, NDV, and/or ELDKWA, essentially as reported (Jordan and Hammond, *supra*). Serial two-fold dilutions of virus preparations, starting at 5µg/ml, were coated directly to ELISA plates. Plates were washed and blocked with 1% BSA in PBS prior to incubation with the appropriate antibody solution, and subsequently reacted with a mix of PTY monoclonal antibodies (cross-reactive; Figures 6A and 6B), with BYMV-specific PTY 24 (Figures 6C and 6D), or with HIV ELDKWA epitope-specific human neutralizing MAb 2F5 (Figures 6E and 6F). Plates were again washed prior to addition of goat anti-mouse (for mix of PTY monoclonal antibodies and for PTY 24), or goat anti-human (for MAb 2F5) alkaline phosphatase conjugate.

The relative reactions of the BYMV preparations from ELDKWA transgenic plants with the PTY mix and PTY 24 are less than the reaction of a control BYMV preparation from non-transgenic plants (Figures 6A-6D). In contrast, the reactions of virus from ELDKWA transgenic plants with HIV-specific MAb 2F5 are significantly higher than the background level reaction of the BYMV from non-transgenic control plants, thus demonstrating the presence of the ELDKWAcp in virions purified from the transgenic plants.

EXAMPLE 8

Stimulation of Epitope-specific Antibodies in Mice Injected with Purified Virions

Mice were injected with purified PVY virions bearing the F epitope of NDV isolated as described previously. Immunizations contained 200 ug of purified virus in 200 ul of

Tris-buffered saline (TBS) emulsified with 240 ul Hunters's TiterMaxGold adjuvant and 100 ul injected in each of four mice. The mice were injected a minimum of 4 times over a 2-3 month time frame with injections 10-14 days apart with a minimum 21 day rest after the third injection. Tail bleeds were 10 - 14 days after the 4th injection. All injections were done intraperitoneally.

Purified recombinant TMV carrying the F epitope on the BYMV CP or inactivated NDV were electrophoresed on a 10% SDS polyacrylamide gel. The proteins were transferred to an Immobilon membrane which was subsequently incubated with the serum derived from mice that had been injected as described above.

The results provide evidence that the NDV F protein epitope presented at the surface of the potyvirus CP could elicit the production of antibodies that react with the F protein of NDV (Figure 7). The positive reaction in the left lane indicates the location of the BYMV-F CP that is translated from the TMV construct in infected plants. The arrow points to the location of the 58,000 Da F protein in inactivated NDV isolated from diseased chickens.

Mice that were injected with the BYMV virions carrying HIV ELDKWA epitope-containing CPs have not been bled.

EXAMPLE 9

Bacterial expression of ELDKWAcp

The HIV epitope/BYMV CP fusion protein ELDKWAcp was also expressed as an in-frame fusion with the *lacZ* α-peptide in *Escherichia coli* from the vector pCR2.1. This was used to confirm the reactivity of the ELDKWAcp protein with the HIV-specific MAb 2F5, and replacement of the BYMV-specific immunodominant epitopes by the ELDKWA epitope. Expression of bacterially-expressed potyvirus CP (wild-type BYMV CP and ELDKWAcp) was enhanced by IPTG induction as previously reported (Hammond and Hammond. 1989. *J. Gen. Virol.* 70:1961-1974). Fifteen µl of extracts of *Escherichia coli* strain DH5α and purified BYMV (strain Ideal A) were electrophoresed through a 10% polyacrylamide gel, and blotted to an Immobilon PVDF membrane. The extracts analyzed were: BYMV-Ideal A (Purified BYMV, isolate Ideal A); DH5α/pCR2.1 (*E. coli* strain DH5α carrying plasmid vector pCR2.1 - negative control #1); DH5α/ELDKWAcp (*E. coli* strain DH5α carrying plasmid pCR2.1/ELDKWAcp, expressing the ELDKWAcp as a *lacZ* fusion protein); DH5α/BYMV-CP (*E. coli* strain DH5α carrying plasmid pBY9, expressing BYMV-GDD CP as a *lacZ* fusion protein); and DH5α (without any plasmid - negative control #2).

Membranes were probed with (a) MAb PTY 43 (specific for BYMV isolate GDD; Jordan and Hammond, *supra*) which reacts with the N-terminal portion of the BYMV-GDD CP (Jordan, 1992, *Arch. Virol.* [Suppl.5]81-95); (b) potyvirus cross-reactive MAb PTY 2 (Jordan and Hammond, *supra*); and (c) HIV-specific MAb 2F5 (Muster *et al.* 1993. *J. Virol.* 67:6642-6647).

MAb PTY 43 reacts only with BYMV-GDD CP, expressed from *E. coli*, and not BYMV-Ideal A. MAb 2F5 reacts only with ELDKWAcp. In contrast, MAb PTY 2 reacts with BYMV-Ideal A, and both bacterially-expressed BYMV-GDD CP and ELDKWAcp. There is also an apparent non-specific activity, with one band present in all DH5α extracts, and one or more bands apparently derived from pCR2.1. The ELDKWAcp band has lower apparent molecular mass than BYMV-CP, as predicted. These experiments demonstrate that the BYMV-GDD specific epitope recognized by MAb PTY 43 had been replaced with the ELDKWA epitope recognized by MAb 2F5, and that the HIV epitope was serologically active (Figure 8).

EXAMPLE 10

Expression of ELDKWAcp from the PVX vector

The ELDKWAcp construct was also expressed from the infectious PVX vector in *N. benthamiana* and *N. tabacum* plants. T7 transcripts were inoculated to young plants, and sap from the systemically-infected plants used to infect additional plants. Indirect, antigen-coated plate ELISA was performed essentially as described by Jordan and Hammond, 1991 (*supra*). Extracts of infected leaves (1:100, w/v) of plants were prepared in coating buffer plus polyvinylpyrrolidone (PVP) plus diethyldithiocarbamic (DIECA) and coated directly to ELISA plates. Following incubation the plates were washed and blocked with 1% BSA in PBS prior to incubation with the appropriate antibody solution (Figure 9A, PVX-specific rabbit polyclonal; Figure 9B, PTY 1 potyvirus cross-reactive mouse MAb; Figure 9C, PTY 24 BYMV-specific mouse MAb; and Figure 9D, 2F5 HIV-ELDKWA-specific human MAb). Plates were again washed prior to addition of goat anti-rabbit (for detection of PVX-specific polyclonal antiserum), goat anti-mouse (for PTY 1 and PTY 24), or goat anti-human (for MAb 2F5) alkaline phosphatase conjugate. Samples were: A, healthy *Nicotiana benthamiana* (negative control); B, BYMV-infected *Nicotiana benthamiana* (BYMV control); C, *Nicotiana benthamiana* plant 1A infected with PVX carrying the ELDKWAcp as an additional gene; and D, *Nicotiana benthamiana* plant 6A infected with PVX carrying the ELDKWAcp as an additional gene.

The results demonstrate the expression of ELDKWAcp from the PVX vector inoculated to non-transgenic plants of *Nicotiana benthamiana* (Figures 9A-9D). Figure 9A (PVX polyclonal) shows that PVX is detected only in the PVX/ELDKWA-inoculated plants. Figure 9C (lower left) shows that the PVX/ELDKWA-inoculated plants are not infected with BYMV, thus confirming that the reactions of samples C and D with MAbs PTY 1 and 2F5 are due to the ELDKWAcp expressed from the PVX vector.

All publications and patents mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

It is understood that the foregoing detailed description is given merely by way of illustration and that modifications and variations may be made therein without departing from the spirit and scope of the invention. Accordingly, the following claims are intended to be interpreted to embrace all such modifications.

We claim:

1. A cDNA construct containing a DNA sequence which encodes a fusion coat protein comprising a truncated potyvirus coat protein and a foreign antigenic peptide.

2. The cDNA construct of claim 1 wherein the truncated potyvirus coat protein is a BYMV coat protein.

3. The cDNA construct of claim 1 or claim 2 wherein the foreign antigenic peptide is selected from the group consisting of a Newcastle Disease Virus (NDV) epitope and a human immunodeficiency virus type 1 (HIV-I) epitope.

4. The cDNA construct of claim 3 wherein the NDV epitope is antigenic determinant II, a continuous epitope consisting of 17 amino acids identified by SEQ ID NO:12.

5. The cDNA construct of claim 3 wherein the HIV-I epitope is the gp41 epitope ELDKWA identified by SEQ ID NO:8.

6. A cDNA construct comprising the DNA sequence identified by SEQ ID NO:10.

7. A cDNA construct comprising a DNA sequence which encodes a fusion coat protein comprising the amino acid sequence identified by SEQ ID NO:11.

8. A cDNA construct comprising the DNA sequence identified by SEQ ID NO:17.

9. A vector containing the construct of any one of claims 1-8.

10. A vector comprising a full length cDNA of PVX mRNA containing a DNA insert encoding a fusion coat protein comprising a truncated BYMV coat protein and foreign antigenic peptide.

11. The vector of claim 10 wherein the foreign antigenic peptide is a NDV epitope.

12. The vector of claim 10 wherein the foreign antigenic peptide is an HIV epitope.

13. A vector comprising a full length cDNA of TMV mRNA containing a DNA insert encoding a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide.

14. The vector of claim 13 wherein the foreign antigenic peptide is an HIV-I epitope.

15. An infectious RNA transcript of the vector of any one of claims 9-14.

16. PVY virions containing foreign antigenic peptide inserts on the exposed surface of the coat protein of the virion, the inserts resulting from a transencapsidation process during virion assembly whereby copies of PVX coat protein containing the fusion coat protein of claim 1 and copies of PVY coat protein assemble to form the PVY capsid.

17. PVY virions containing foreign antigenic peptide inserts on the exposed surface of the coat protein of the virion, the inserts resulting from a transencapsidation process during virion assembly whereby copies of PVX coat protein containing the fusion coat protein of claim 2 and copies of PVY coat protein assemble to form the PVY capsid.

18. An immunogenic composition comprising the PVY virions of claim 16 and a carrier.

19. An immunogenic composition comprising the PVY virions of claim 17 and a carrier.

20. An immunogenic composition as in claim 18 or claim 19 wherein the carrier is a diluent or an edible part of a plant infected with the PVY virions.

21. A method for producing a foreign antigenic peptide in a plant comprising:
    a) infecting a host plant with PVY virions;
    b) inoculating the PVY-infected host plant with the infectious RNA of claim 15;
    c) culturing the plant under conditions to foster plant growth and transencapsidation; and
    d) recovering the transencapsidated PVY virions from the leaves of the plant.

22. The method of claim 21 wherein the host plant is selected from the group consisting of lettuce, spinach, tomato, potato, *Nicotiana tabacum*, *N. glutinosa*,

N. sylvestris, N. benthamiana, Phaseolus vulgaris, and *Chenopodium amaranticolor.*

23. A method for producing an immune response stimulating peptide in a plant comprising:
    a) infecting a host plant with PVY virions;
    b) inoculating the PVY-infected host plant with the infectious RNA of claim 15;
    c) culturing the plant under conditions to foster plant growth and transencapsidation; and
    d) recovering the immune response stimulating peptide from the leaves of the plant.

24. A transgenic plant expressing a nucleotide sequence which encodes a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide.

25. A plasmid vector for transforming a plant comprising:
    a DNA sequence encoding a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide; and
    a plant-functional promoter operably linked to said DNA sequence capable of directing the expression of said DNA sequence in said plant.

26. The plasmid vector of claim 25 wherein said plant promoter comprises CaMV35S.

27. A method for constructing a transgenic plant cell comprising:
    constructing a DNA vector by operably linking a DNA sequence encoding a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide to a plant-functional promoter capable of directing the expression of said DNA sequence in said plant; and
    transforming a plant cell with said DNA vector.

28. A method for producing a pharmaceutical vaccine composition, wherein the pharmaceutical vaccine composition consists of a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide, comprising the steps of:
    constructing a DNA vector by operably linking a DNA sequence encoding said fusion coat protein comprising a truncated BYMV coat protein

and a foreign antigenic peptide, to a plant-functional promoter capable of directing the expression of said DNA sequence in a plant;

transforming a plant with said DNA vector; and

recovering said pharmaceutical vaccine composition expressed in said plant.

29. A vaccine composition comprising:

a fusion coat protein comprising a truncated BYMV coat protein and a foreign antigenic peptide;

and plant material, wherein said vaccine composition is capable of eliciting an immune response upon administration to an animal.

30. A transgenic plant comprising the recombinant virion of claim 16 or claim 17.

31. An oligonucleotide primer selected from the group consisting of the sequence 5'-GAAGGAAATCCTAATGAGCTCGATAAGTGGGCAAGTGTCAGGCAAATAGTACC-3' (SEQ ID NO:1), 5'-CTTTTTCCTTTTATCGAGCTCATTTGACCATGCATTGAGTTGCTC-TTGATCTGC-3' (SEQ ID NO:2), 5'-GATTACGCCAAGCTTTAAAACAATGGCAGATCA-AGAGCAACTCAATGC-3' (SEQ ID NO:4), 5'-CCCAAGCTTAATTAATACAATGGCA-GATCAAGAGCAATTGTTGCC-3' (SEQ ID NO:13), 5'-TTTGCGCATGCTTCCTTATCC-TTTGGCATATTTGGCAACAATTGCTCTTG-3' (SEQ ID NO:14); 5'-GGAAGCATGCGC-AAAGGCACCATTGGTCAGGCAAATAGTACCA-3' (SEQ ID NO:15), and 5'-GGA-ATTCTCGAGCTAAATACGAACACCAAGCA-3' (SEQ ID NO:16).

32. A primer set comprising oligonucleotides consisting of the sequences 5'-CCCAAG-CTTAATTAATACAATGGCAGATCAAGAGCAATTGTTGCC-3' (SEQ ID NO: 13) and 5'-TTTGCGCATGCTTCCTTATCCTTTGGCATATTTGGCAACAATTGCTCTTG-3' (SEQ ID NO:14).

33. A primer set comprising oligonucleotides consisting of the sequences 5'-GGAAGC-ATGCGCAAAGGCACCATTGGTCAGGCAAATAGTACCA-3' (SEQ ID NO:15) and 5'-GGAATTCTCGAGCTAAATACGAACACCAAGCA-3' (SEQ ID NO:16).

34. A primer set comprising oligonucleotides consisting of the sequences 5'-CTTTTT-CCTTTTATCGAGCTCATTTGACCATGCATTGAGTTGCTCTTGATCTGC-3' (SEQ ID NO:2) and 5'-GATTACGCCAAGCTTTAAAACAATGGCAGATCAAGAGCAACTCAA-TGC-3' (SEQ ID NO:4).

35. A primer set comprising oligonucleotides consisting of the sequence 5'-GAAGGA-AATCCTAATGAGCTCGATAAGTGGGCAAGTGTCAGGCAAATAGTACC-3' (SEQ ID NO:1) and the M13F primer.

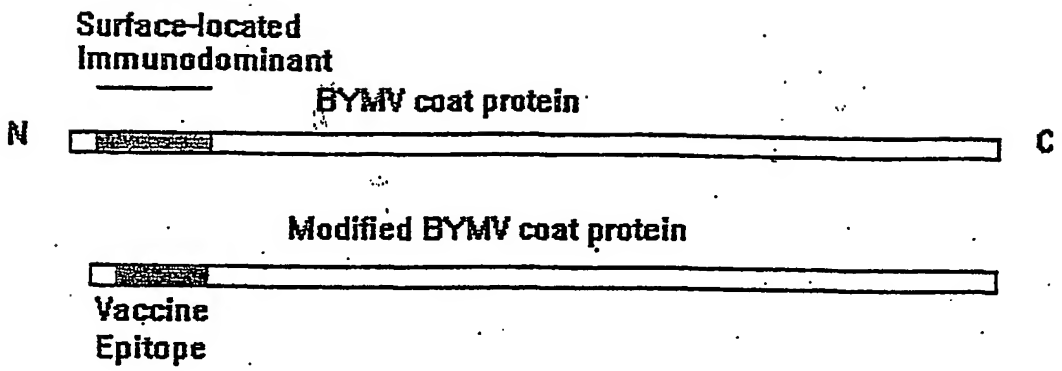

Surface-located
Immunodominant
BYMV coat protein
N
C
Modified BYMV coat protein
Vaccine
Epitope

FIG. 1a

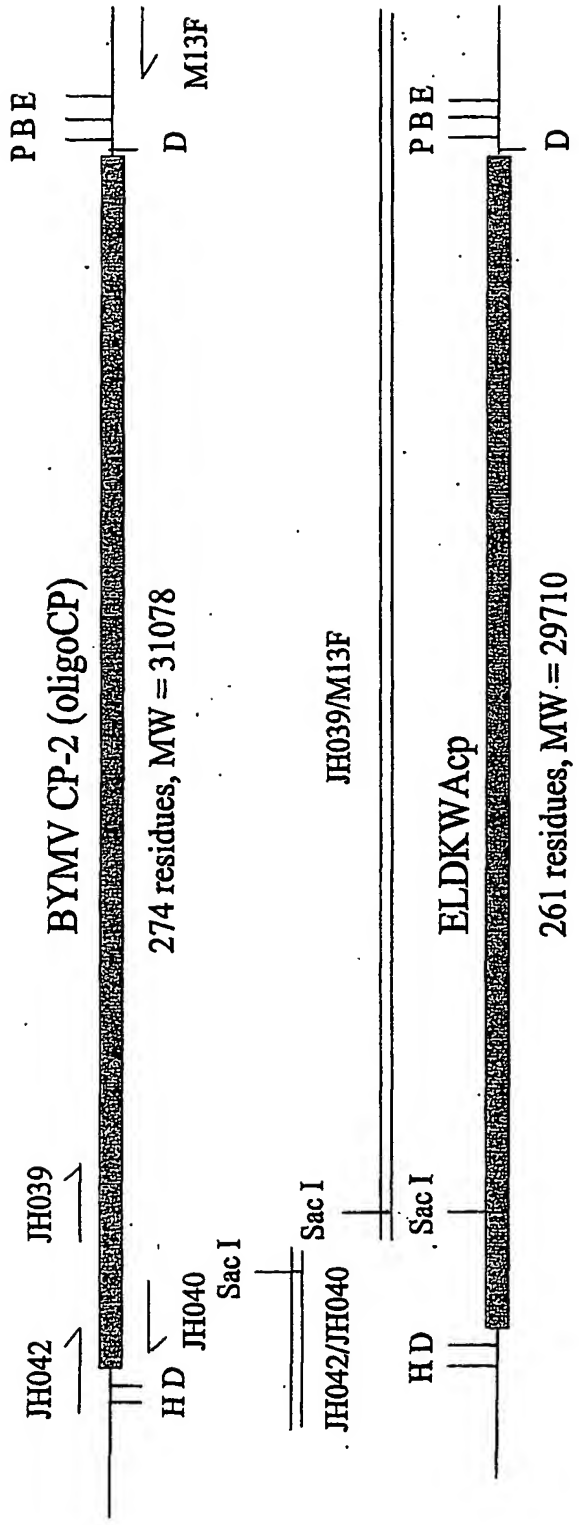

P B E
D
M13F
BYMV CP-2 (oligoCP)
274 residues, MW = 31078
JH039
JH042
H D
JH040
Sac I
Sac I
JH042/JH040
JH039/M13F
P B E
D
ELDKWAcp
261 residues, MW = 29710
Sac I
H D

FIG. 1b

(1) Forward primer JH039

JH039 5'-GAAGGAAATCCTAATGAGCTCGATAAGTGGGCAAGTGTCAGGCAAATAGTACC-3'(SEQ ID NO:1)

```
   M  A  D  Q  E  Q  L  N  A  G  E  E  K  K  D  K  R  K  K  N  E  G  N  P  N  K  D  S  E
5'ATGGCAGATCAAGAGCAACTCAATGCAGGTGAGGAGAAGAAGGATAAAAGGAAAAAGAATGAAGGAAATCCTAATAAGGACTCTGAG
                                                                5'GAAGGAAATCCTAATGAGCTCGATAAG
                                                                      E  L  D  K
                                                                     Sac I
 G  Q  S  V  R  Q  I  V      (SEQ ID NO:7)
GGGCAGAGTGTCAGGCAAATAGTACC 3'(SEQ ID NO:6)
TGGGCAAGTGTCAGGCAAATAGTACC 3'(SEQ ID NO:1)
 W  A     (SEQ ID NO:8)
```

FIG. 1c

(2) Reverse primer JH040 is the complement of SEQ ID NO:3

```
5'GCAGATCAAGAGCAACTCAATGCATGGTCAAATGAGCTCGATAAAAGGAAAAAG 3'(SEQ ID NO:3)
3'CGTCTAGTTCTCGTTGAGTTACGTACCAGTTTACTCGAGCTATTTTCCTTTTTC 5'(Complement 3'→5')
```

```
Reverse Primer JH040(5'→3'):
5'CTTTTTCCTTTTATCGAGCTCATTTGACCATGCATTGAGTTGCTCTTGATCTGC 3'(SEQ ID NO:2)
```

```
   M  A  D  Q  E  Q  L  N  A  G  E  E  K  K  D  K  R  K  K  N  E  G  N  P  N  K  D  S  E
5'ATGGCAGATCAAGAGCAACTCAATGCAGGTGAGGAGAAGAAGGATAAAAGGAAAAAGAATGAAGGAAATCCTAATAAGGACTCTGAG
   5'GCAGATCAAGAGCAACTCAATGCATGGTCAAATGAGCTCGATAAAAGGAAAAAG 3'(SEQ ID NO:3)
                              W  S  N  E  L  D  (SEQ ID NO:9)
                                       Sac I

 G  Q  S  V  R  Q  I  V       (SEQ ID NO:7)
GGGCAGAGTGTCAGGCAAATAGTACC 3'(SEQ ID NO:6)
```

(3) Forward primer JH042

```
    I  T  P  S  F  K  T  M  A  D  Q  E  Q  L  N  A   (SEQ ID NO:5)
5'GATTACGCCAAGCTTTAAAACAATGGCAGATCAAGAGCAACTCAATGC 3'  (SEQ ID NO:4)
```

Hind III

DRA I

FIG. 1c (cont.)

M A D Q E Q L N A G E E K K D K R K K N
5'ATGGCAGATCAAGAGCAACTCAATGCAGGTGAGGAGAAGAAGGATAAAAGGAAAAAGAAT

Complement of JH040: (SEQ ID NO:3)
5'GCAGATCAAGAGCAACTCAATGCATGGTCAAATGAGCTCGATAAAAGGAAAAAG 3'

W S N E L D (SEQ ID NO:9)

Sac I

E G N P N K D S E G Q S V R Q I V
GAAGGAAATCCTAATAAGGACTCTGAGGGGCAGAGTGTCAGGCAAATAGTACC 3'

Primer JH039: (SEQ ID NO:1)
5'GAAGGAAATCCTAATGAGCTCGATAAGTGGGCAAGTGTCAGGCAAATAGTACC 3'

E L D K W A (SEQ ID NO:8)

Sac I

ELDKWA epitope-modified BYMV CP amino-terminal sequence:

M A D Q E Q L N A W S N E L D K W A S V R
5'ATGGCAGATCAAGAGCAACTCAATGCATGGTCAAATGAGCTCGATAAGTGGGCAAGTGTCAGG
Sac I

Q I V (SEQ ID NO:11)
CAAATAGTACC 3' (SEQ ID NO:10)

FIG. 1d

I T P S F K T M A D Q E Q L N A (SEQ ID NO:5)
5'GATTACGCCAAGCTTTAAAACAATGGCAGATCAAGAGCAACTCAATGC 3' (SEQ ID NO:4)

(SEQ ID NO:3) GCAGATCAAGAGCAACTCAATGCATGGTCAAATGAGCTCGATAAAAGGAAAAAG
(SEQ ID NO:9) W S N E L D

FIG. 1e

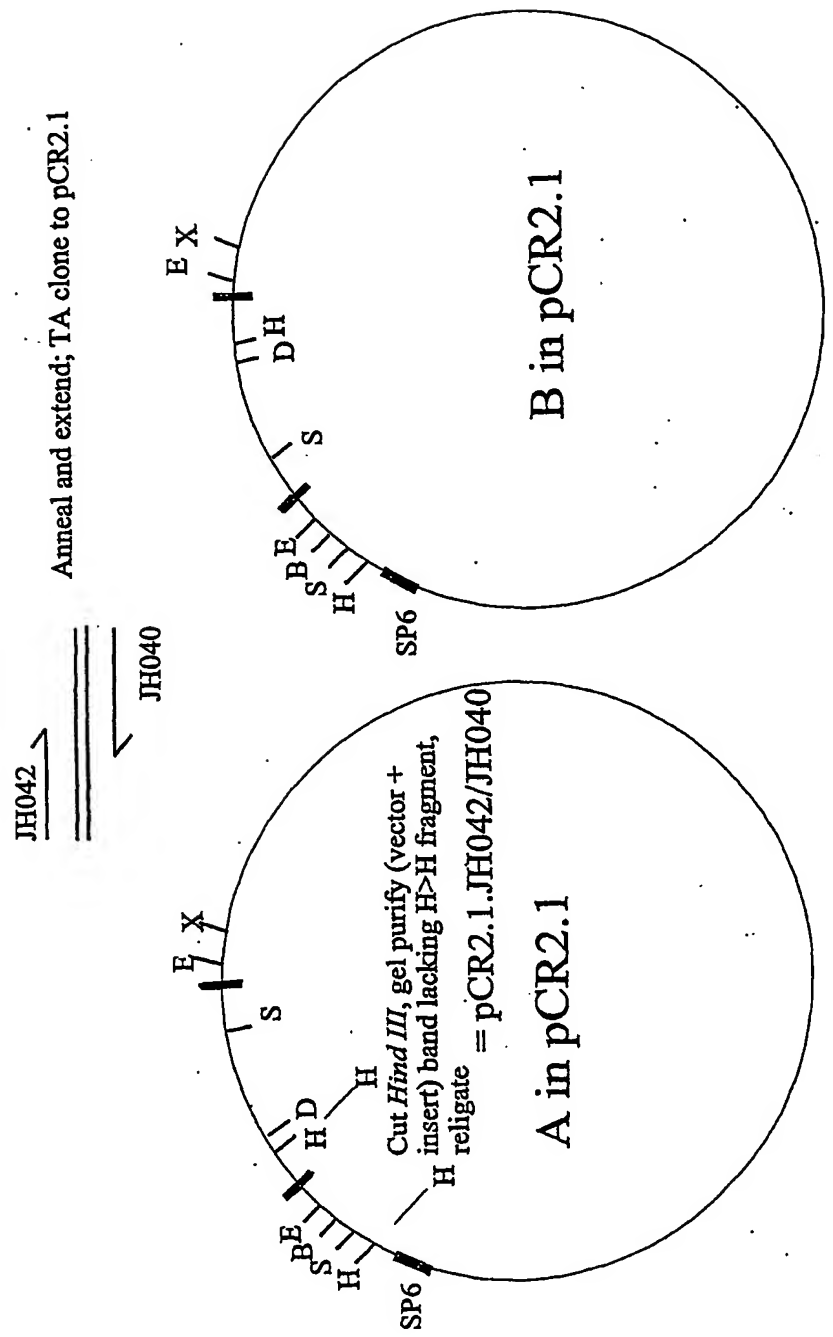

JH042
JH040
Anneal and extend; TA clone to pCR2.1
A in pCR2.1
B in pCR2.1
Cut Hind III, gel purify (vector + insert) band lacking H>H fragment, religate
= pCR2.1.JH042/JH040
E X
S
H D
H
B E
S
H
SP6
E X
D H
S
B E
S
H
SP6

FIG. 1f

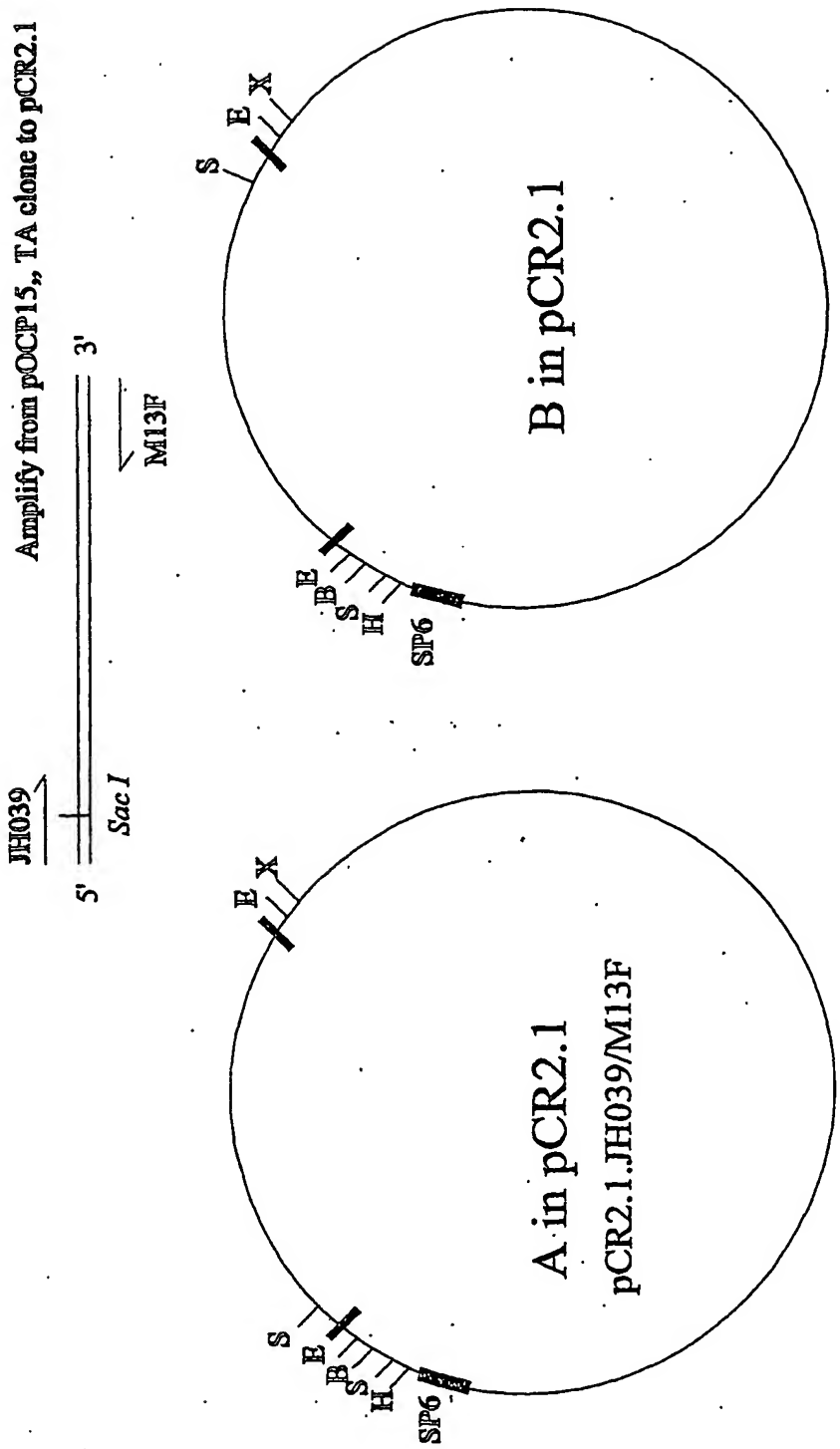

Amplify from pOCP15„ TA clone to pCR2.1
JH039
5'
Sac I
3'
M13F
A in pCR2.1
pCR2.1.JH039/M13F
B in pCR2.1
E
X
S
B
H
SP6

FIG. 1g

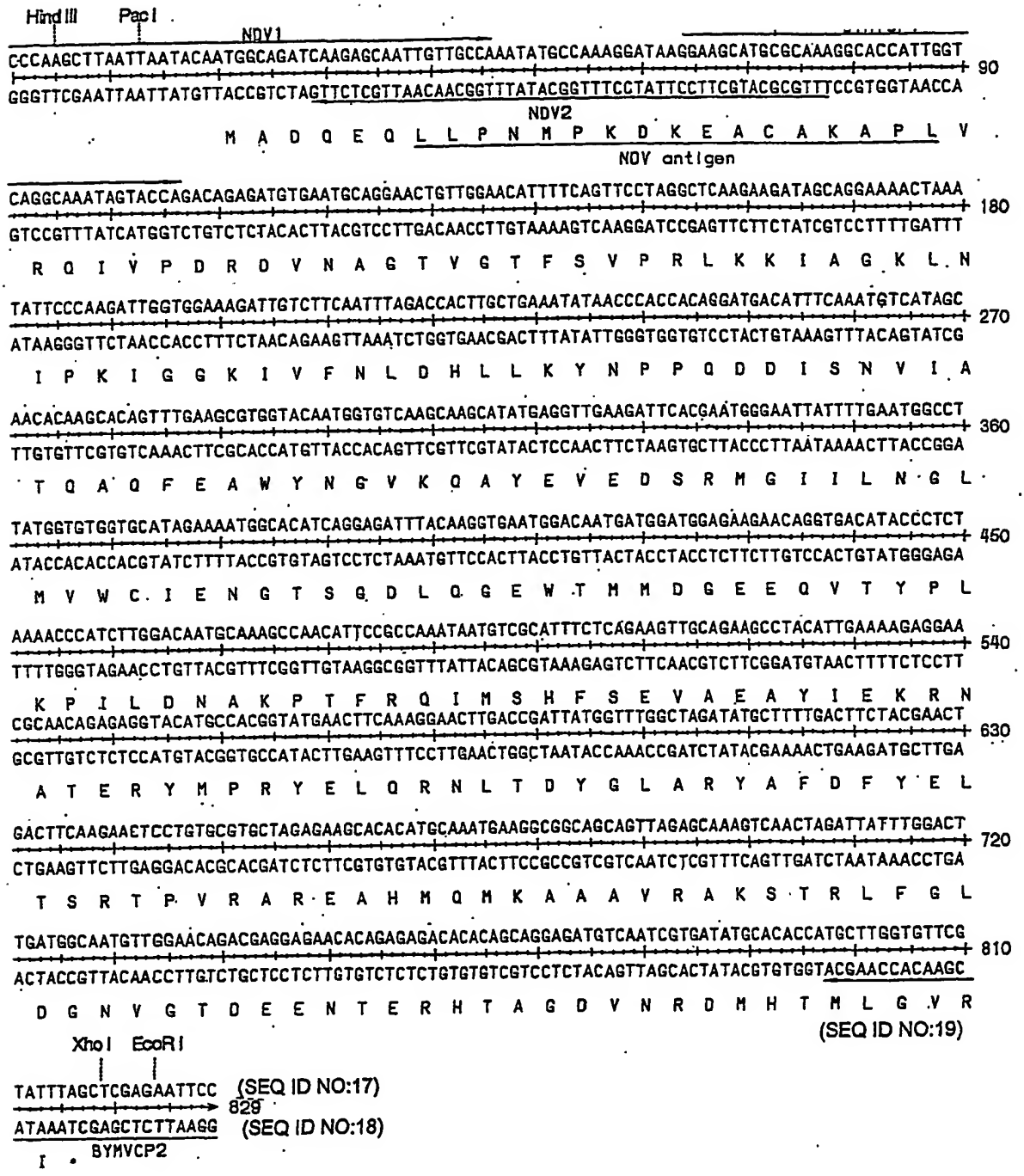

Hind III
Pac I
NDV1
NDV2
NDV antigen
Xho I
EcoR I
(SEQ ID NO:19)
(SEQ ID NO:17)
(SEQ ID NO:18)
BYMVCP2

FIG. 2

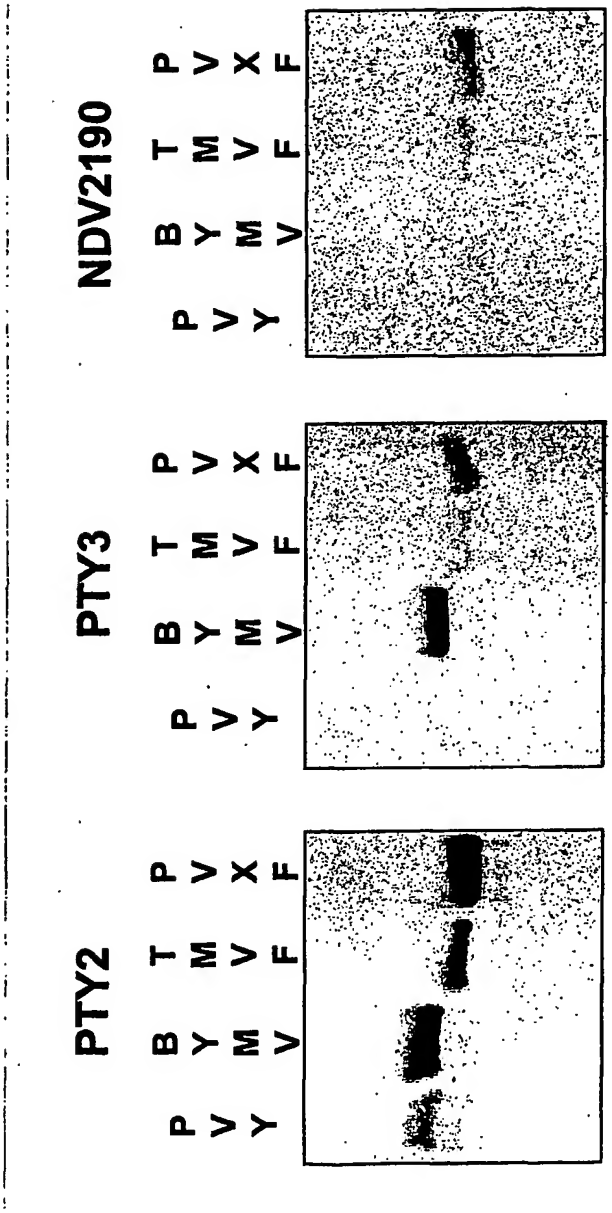
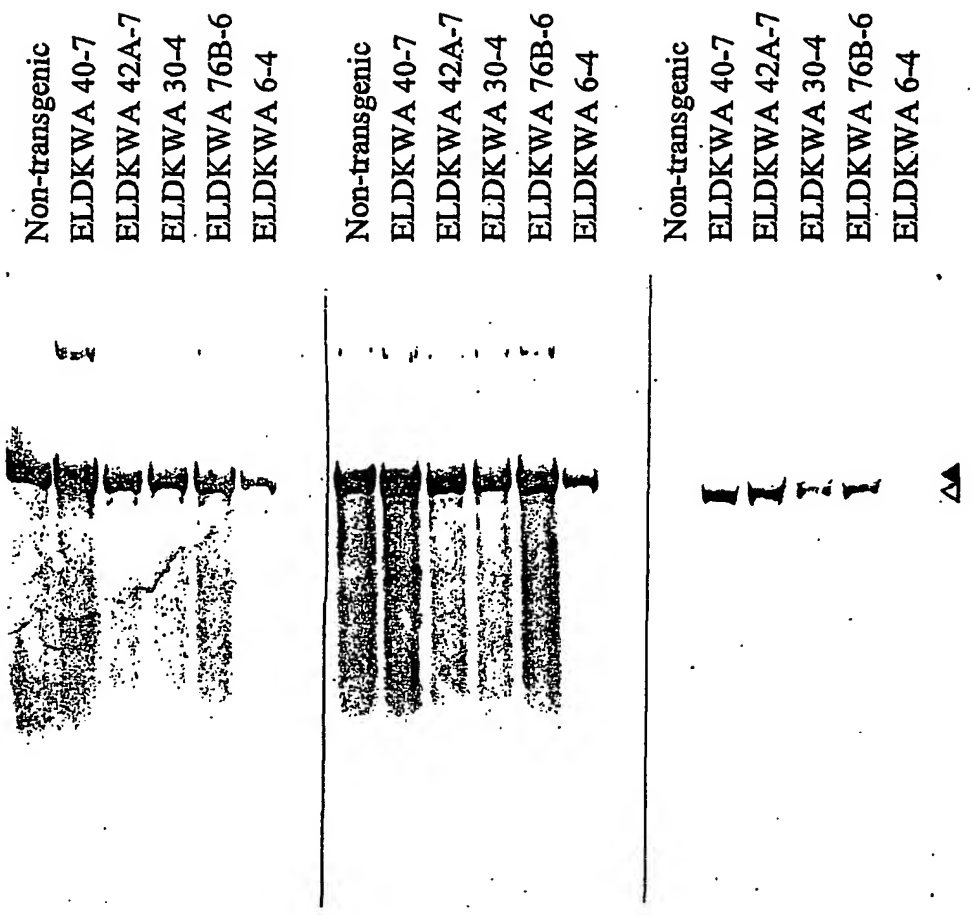


FIG. 3A FIG. 3B FIG. 3C

FIG. 3



PTY mix
cross-reactive

PTY 24
BYMV-specific

2F5
ELDKWA-specific

FIG. 4A FIG. 4B FIG. 4C

FIG. 4

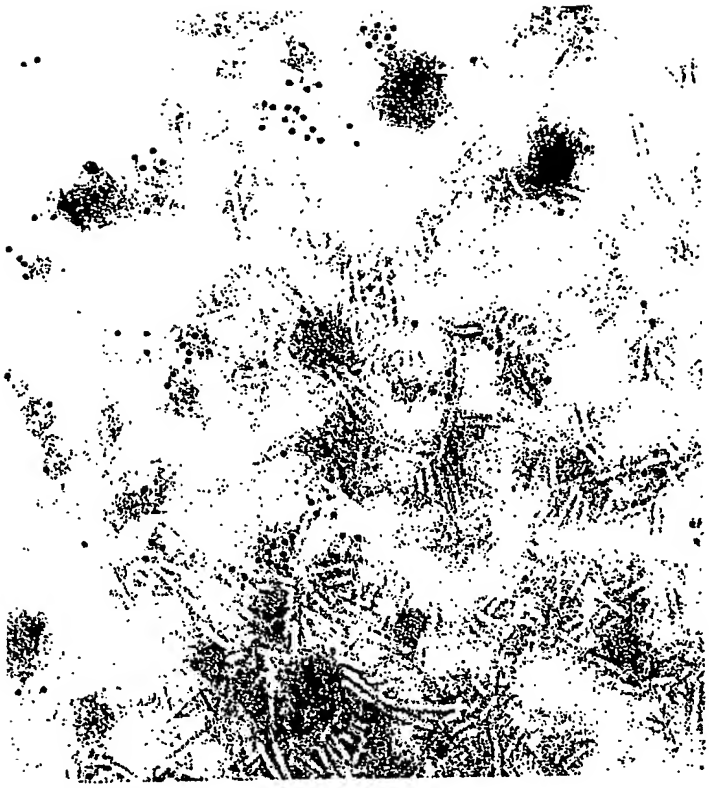


FIG. 5

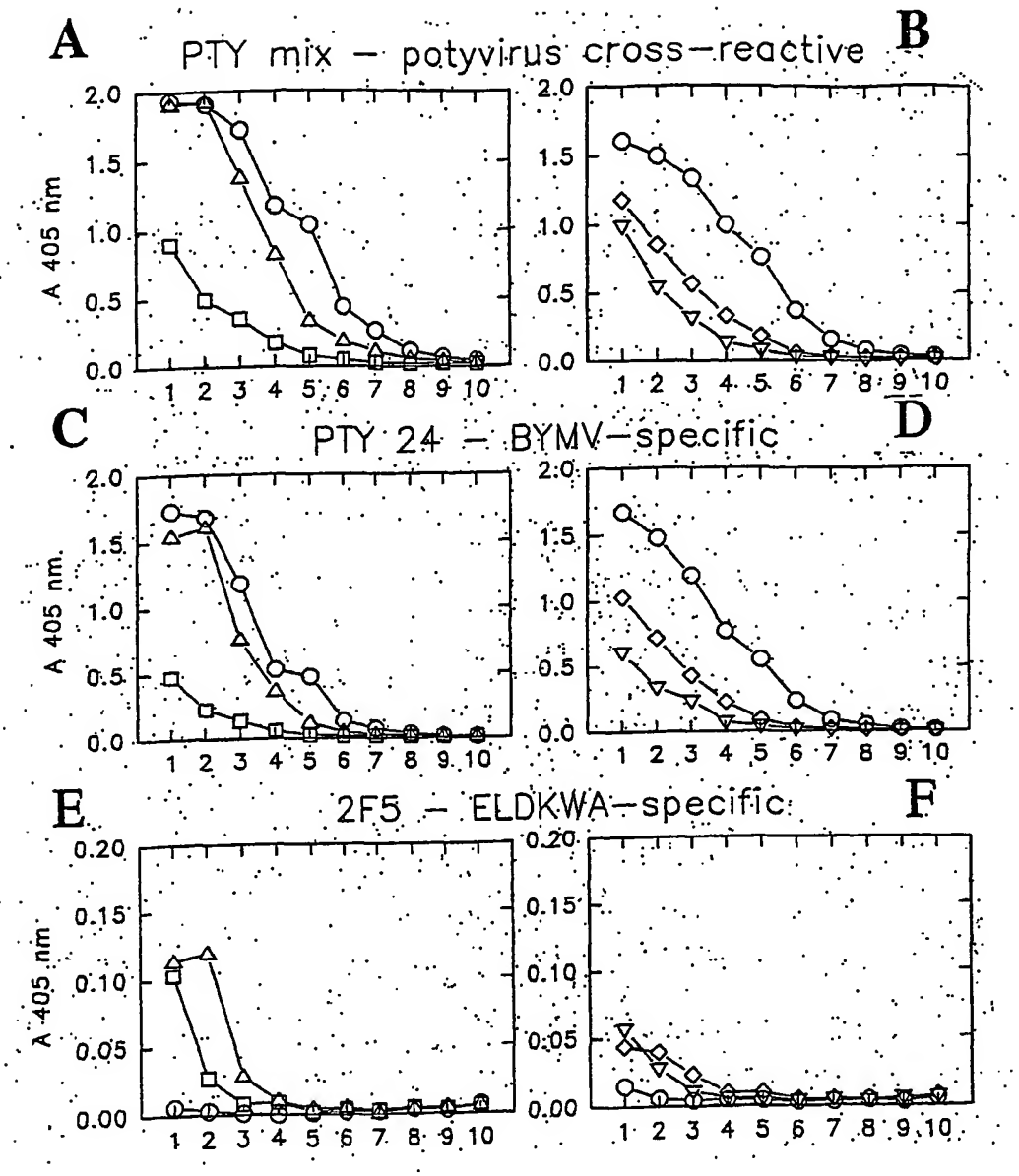

A
PTY mix – potyvirus cross–reactive
B
C
PTY 24 – BYMV–specific
D
E
2F5 – ELDKWA–specific
F
A 405 nm
2.0
1.5
1.0
0.5
0.0
0.20
0.15
0.10
0.05
0.00
1 2 3 4 5 6 7 8 9 10

FIG. 6

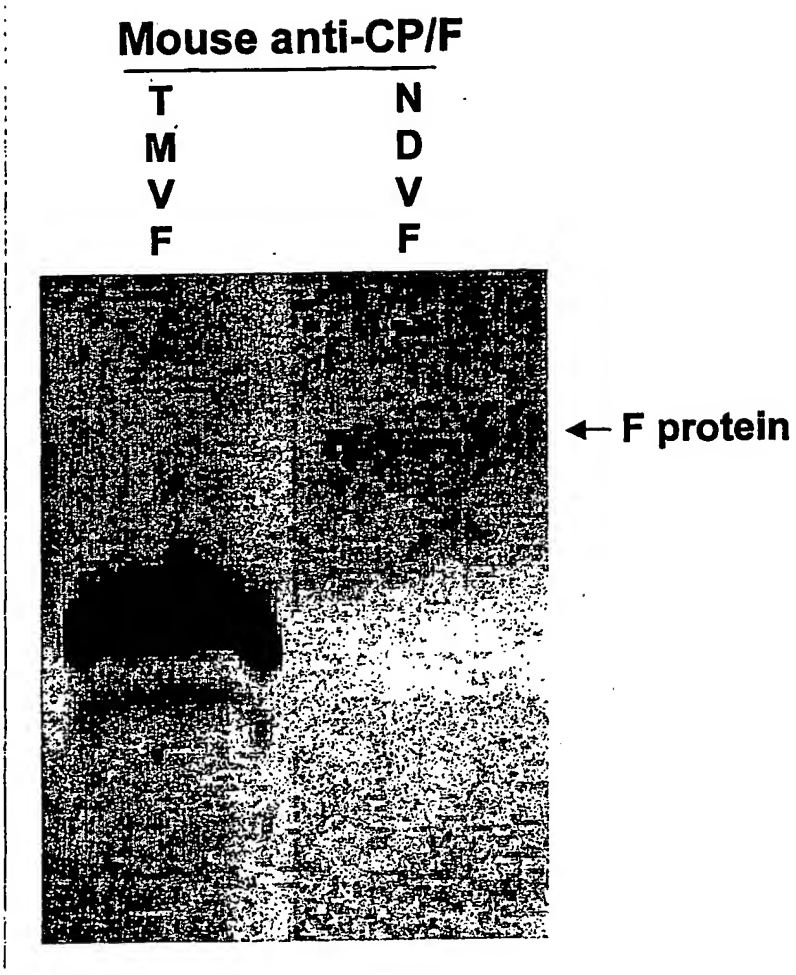


FIG. 7

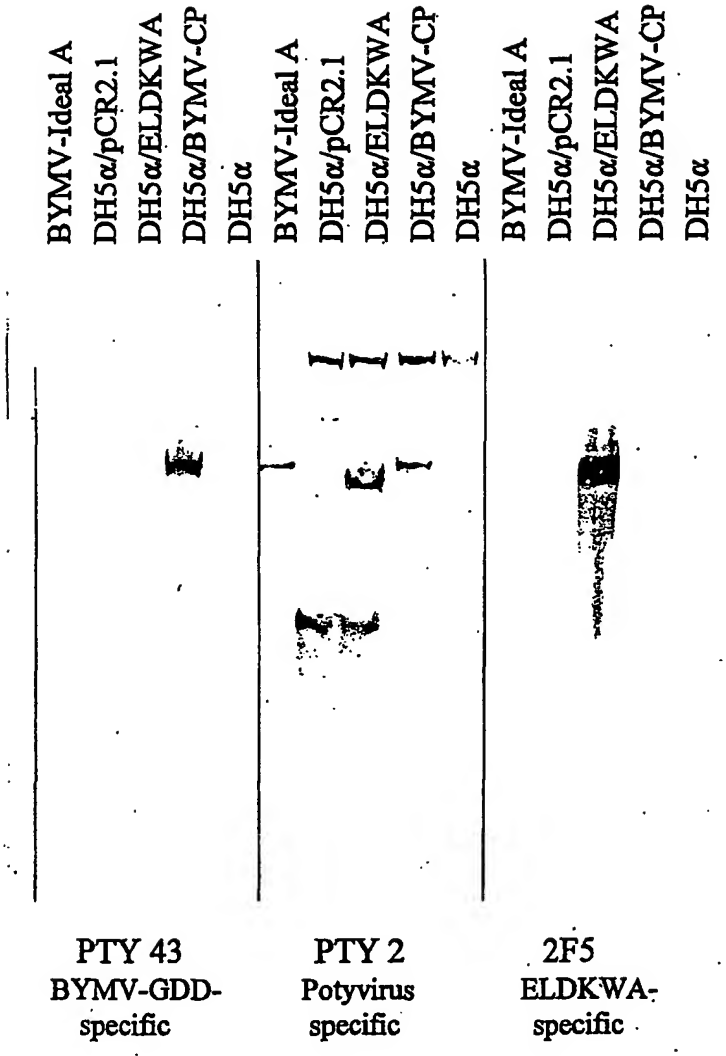

BYMV-Ideal A
DH5α/pCR2.1
DH5α/ELDKWA
DH5α/BYMV-CP
DH5α
BYMV-Ideal A
DH5α/pCR2.1
DH5α/ELDKWA
DH5α/BYMV-CP
DH5α
BYMV-Ideal A
DH5α/pCR2.1
DH5α/ELDKWA
DH5α/BYMV-CP
DH5α
PTY 43
BYMV-GDD-specific
PTY 2
Potyvirus specific
2F5
ELDKWA-specific

FIG. 8

FIG. 9A

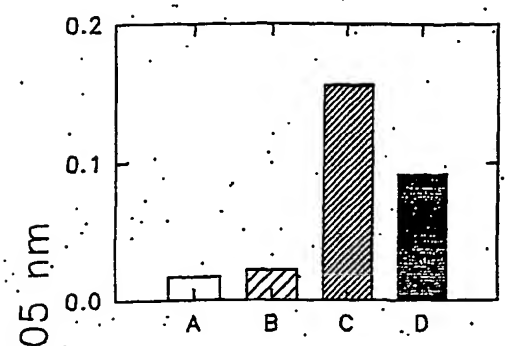


FIG. 9B

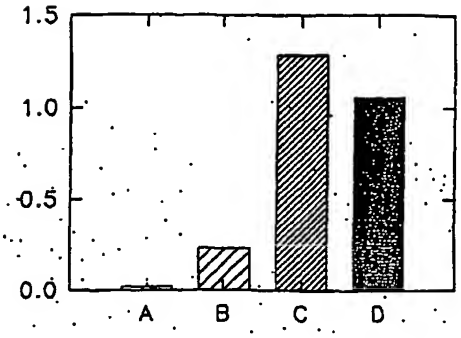


FIG. 9C

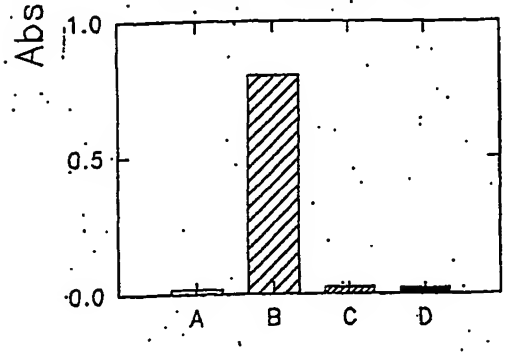


FIG. 9D

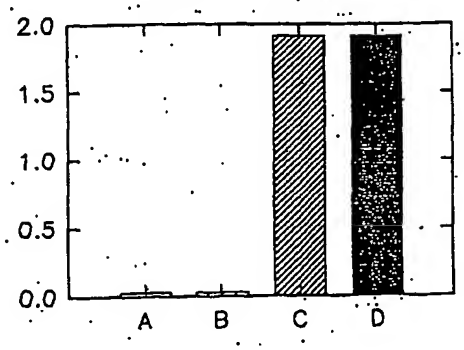


FIG. 9/16

SEQUENCE LISTING

<110> Hammond, Rosemarie
Zhao, Yan
Hammond, John

<120> Production of Vaccines Using Transgenic Plants or Modified Plant Viruses as Expression Vectors and Transencapsidated Viral Coat Proteins as Epitope Presentation Systems

<130> 0153.97

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1 5 10 15

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1 5 10 15

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20 25 30

Val Arg Gln Ile Val
35

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Glu Leu Asp Lys Trp Ala
1 5

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Trp Ser Asn Glu Leu Asp
1 5

<210> 10
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atggcagatc aagagcaact caatgcatgg tcaaatgagc tcgataagtg ggcaagtgtc 60
aggcaaatag tacc 74

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<211> 24
<212> PRT
<213> bean yellow mosaic virus

<400> 11
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1 5 10 15

Trp Ala Ser Val Arg Gln Ile Val
20

<210> 12
<211> 17
<212> PRT
<213> Newcastle disease virus

<400> 12
Leu Leu Pro Asn Met Pro Lys Asp Lys Glu Ala Cys Ala Lys Ala Pro
1 5 10 15

Leu

<210> 13
<211> 45
<212> DNA
<213> Newcastle disease virus

<400> 13
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<210> 14
<211> 50
<212> DNA
<213> Newcastle disease virus

<400> 14
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<210> 15
<211> 43
<212> DNA
<213> bean yellow mosaic virus

<400> 15
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<210> 16
<211> 32
<212> DNA
<213> bean yellow mosaic virus

<400> 16
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<210> 17
<211> 829
<212> DNA
<213> bean yellow mosaic virus

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tattcccaag attggtggaa agattgtctt caatttagac cacttgctga aatataaccc 240
accacaggat gacatttcaa atgtcatagc aacacaagca cagtttgaag cgtggtacaa 300
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agcacacatg caaatgaagg cggcagcagt tagagcaaag tcaactagat tatttggact 720
tgatggcaat gttggaacag acgaggagaa cacagagaga cacacagcag gagatgtcaa 780
tcgtgatatg cacaccatgc ttggtgttcg tatttagctc gagaattcc 829

<210> 18
<211> 829
<212> DNA
<213> bean yellow mosaic virus

<400> 18
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acgtccttga caaccttgta aaagtcaagg atccgagttc ttctatcgtc cttttgattt 180
ataagggttc taaccacctt tctaacagaa gttaaatctg gtgaacgact ttatattggg 240
tggtgtccta ctgtaaagtt tacagtatcg ttgtgttcgt gtcaaacttc gcaccatgtt 300
accacagttc gttcgtatac tccaacttct aagtgcttac ccttaataaa acttaccgga 360
ataccacacc acgtatcttt taccgtgtag tcctctaaat gttccactta cctgttacta 420
cctacctctt cttgtccact gtatgggaga ttttgggtag aacctgttac gtttcggttg 480
taaggcggtt tattacagcg taaagagtct tcaacgtctt cggatgtaac ttttctcctt 540
gcgttgtctc tccatgtacg gtgccatact tgaagtttcc ttgaactggc taataccaaa 600
ccgatctata cgaaaactga agatgcttga ctgaagttct tgaggacacg cacgatctct 660
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actaccgtta caaccttgtc tgctcctctt gtgtctctct gtgtgtcgtc ctctacagtt 780
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<210> 19
<211> 265
<212> PRT
<213> bean yellow mosaic virus

<400> 19

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Val Asn Ala Gly Thr Val Gly Thr Phe Ser Val Pro Arg Leu Lys Lys
        35                  40                  45

Ile Ala Gly Lys Leu Asn Ile Pro Lys Ile Gly Gly Lys Ile Val Phe
    50                  55                  60

Asn Leu Asp His Leu Leu Lys Tyr Asn Pro Pro Gln Asp Asp Ile Ser
65                  70                  75                  80

Asn Val Ile Ala Thr Gln Ala Gln Phe Glu Ala Trp Tyr Asn Gly Val
                85                  90                  95

Lys Gln Ala Tyr Glu Val Glu Asp Ser Arg Met Gly Ile Ile Leu Asn
            100                 105                 110

Gly Leu Met Val Trp Cys Ile Glu Asn Gly Thr Ser Gly Asp Leu Gln
        115                 120                 125

Gly Glu Trp Thr Met Met Asp Gly Glu Glu Gln Val Thr Tyr Pro Leu
    130                 135                 140

Lys Pro Ile Leu Asp Asn Ala Lys Pro Thr Phe Arg Gln Ile Met Ser
145                 150                 155                 160

His Phe Ser Glu Val Ala Glu Ala Tyr Ile Glu Lys Arg Asn Ala Thr
                165                 170                 175

Glu Arg Tyr Met Pro Arg Tyr Glu Leu Gln Arg Asn Leu Thr Asp Tyr
            180                 185                 190

Gly Leu Ala Arg Tyr Ala Phe Asp Phe Tyr Glu Leu Thr Ser Arg Thr
        195                 200                 205

Pro Val Arg Ala Arg Glu Ala His Met Gln Met Lys Ala Ala Ala Val
    210                 215                 220

Arg Ala Lys Ser Thr Arg Leu Phe Gly Leu Asp Gly Asn Val Gly Thr
225                 230                 235                 240

Asp Glu Glu Asn Thr Glu Arg His Thr Ala Gly Asp Val Asn Arg Asp
                245                 250                 255

Met His Thr Met Leu Gly Val Arg Ile
            260                 265
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